An unusual habitat for a common shark: life history, ecology and demographics of the spiny dogfish (*Squalus acanthias*) in Macquarie Harbour, Tasmania

by
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INSTITUTE FOR MARINE & ANTARCTIC STUDIES

STATEMENTS AND DECLARATIONS

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ABSTRACT

Sharks and their relatives (Elasmobranchii) are commonly understood to have a conservative life history that makes them particularly vulnerable to external pressures. However, species with high mobility potential and generalist diets have a higher ecological flexibility, resulting in an increased adaptive potential. One such species is the small and abundant spiny dogfish, *Squalus acanthias.* Intense fishing pressure across much of their northern hemisphere distribution has resulted in the collapse of some populations. Some of these populations have been able to recover from near catastrophic declines after regulatory action was set in place. Due to differences in environment, prey and demographics, the life-history parameters of different populations are variable, thus, appropriate management is highly reliant on apposite information. However, despite being abundant in temperate waters of Australia, the life history of *S. acanthias* in the region has remained largely unstudied.

Squalus acanthias is arguably one of the best-understood elasmobranch species, it is widely distributed and has a conservative life history that closely resembles that of large pelagic shark species. These characteristics make it an ideal model species for the group, especially when the environment, ecology or biology of a population differ from what is typical for the species. One such instance potentially exists in Macquarie Harbour, Tasmania. *Squalus acanthias* inhabits this small (275 km^2) ria estuary where adults of both sexes are found throughout the year, an atypical behaviour for this migratory species. The physicochemical characteristics of the system create a strongly stratified environment that differs from the coastal and oceanic habitats where *S. acanthias* is usually found. Anthropogenic impacts from mine dewatering, changed river flows associated with hydro-electricity production and, more recently, salmonid aquaculture, have been linked to important environmental changes and degradation in the area (e.g. declines in dissolved oxygen levels), which may affect the native fauna. Recognizing the interesting set of circumstances, and the need for current information on the species, the main objective of this study was to conduct a comprehensive investigation into the life-history characteristics, ecology

and biology of *S. acanthias* in Macquarie Harbour to provide information on the status of the species in the area and inform future management.

The first component of this study aimed to use passive acoustic telemetry to investigate the spatial ecology and residency patterns of *S. acanthias* in Macquarie Harbour. Adult male and female *S. acanthias* remained resident within Macquarie Harbour throughout the entirety of a 1.5-year observation period, with no evidence to indicate emigration out of the estuarine system. Individuals showed continuous movement throughout the harbour, with the potential to transverse the whole extent of the system within a few hours. Male and female ranges overlapped throughout the year, although the upper reaches of the estuary were used exclusively by males during winter. These results suggest that *S. acanthias* remain resident in Macquarie Harbour for extended periods, independent of sex or season. Other populations of *S. acanthias* seasonally limit their use of small coastal embayments and typically migrate offshore seasonally, where the spatial distribution of the species is specific to sex and ontogeny. The range-restricted spatial utilisation in Macquarie Harbour will likely increase the population's vulnerability to external stressors, particularly by increasing their susceptibility to environmental degradation.

By implementing three distinct approaches to the modelling of telemetry data, this work developed a robust framework for interpretation of the tracking data set. Random walk models, social network analysis and state-transition models were used complementarily to detect complex trends in the movement and behaviour of a species through time. Site selectivity of *S. acanthias* was found to be non-random, with all individuals showing affinity for the central area of the harbour. Results also showed that although the small area of the harbour prevented sexual segregation, behavioural avoidance resulted in decreased association between sexes during winter. This is the first time such a mechanism has been shown in the species, suggesting

alternative adaptive responses through which species may adjust to density dependent or niche constraints when spatial partitioning is not possible.

The second component of this study sought to investigate the life history of the population, specifically diet, reproduction, age and growth. *Squalus acanthias* are typically opportunistic, generalist feeders; however, in the naturally depauperate Macquarie Harbour, prey composition was overwhelmingly dominated by spill-over pellets from the aquaculture operations. Stomach content analysis and stable isotope mixing models produced consistent results, revealing that the dietary trends seen for the population are seasonally invariant. Alternative prey items were consumed during individual sampling events, the most common being escaped Atlantic salmon and mytilid bivalves, with both also being associated with aquaculture operations. Over the observation period, there was an atypically high prevalence of empty stomachs (68%) suggesting there are no viable prey alternatives to the resources provisioned by aquaculture, which may now act as a limiting factor for the population.

In contrast with other *S. acanthias* populations, reproduction in Macquarie Harbour did not conform to a seasonal pattern, with evidence of continuous mating through the year. Females with candled embryos *in-utero* were observed during all seasons, however, other stages of pregnancy were mostly absent. A high frequency of non-gestational mature females showing no sign of recent pregnancy indicates a resting period between pregnancies. Although the possibility that gestation and parturition occur outside Macquarie Harbour cannot be fully discounted, evidence reported in this study suggests that it is highly unlikely. Furthermore, the sizes and ages at maturation, birth and longevity are some of the lowest reported for the species, contrasted by comparably high juvenile growth rates. Current developments for modelling of life-history characteristics in data-poor stocks were incorporated into the analytical framework implemented in this thesis. The results of which are believed to represent the best available estimates for all demographic parameters while characterizing uncertainty in a way that better

resembles biological variability. Results are suggestive of a population that is reproductively compromised, likely reducing their productivity potential and thus, their ability for recovery.

Overall, this thesis reveals a unique set of behavioural and life-history characteristics in this common elasmobranch that is living in an unusual environment. Their demographic characteristics and spatial ecology strongly suggest a closed or semi-closed population likely isolated within Macquarie Harbour. Epigenetics, environmental change, density dependent responses and anthropogenic pressures are all potential causes of the peculiar life history of this population, however, the absence of historical data precludes the partitioning of these factors. A demographic modelling framework constructed around the results of this work revealed that under current conditions the population is unlikely to thrive and will have a severely limited ability to cope with further external pressures. Thus, the past and ongoing anthropogenic impacts of mining and aquaculture are of concern, as they may have overwhelming effects on the population. Nonetheless, by removing large portions of waste pellets from the system, *S. acanthias* provides an important ecosystem service, likely aiding in the partial mitigation of the effects that spill-over aquaculture feed has on other species and nutrient enrichment of the ecosystem. Also, due to the strong interdependence between aquaculture and the species, *S. acanthias* could serve as an ideal indicator species to evaluate the condition of Macquarie Harbour. Considering the results of this thesis, it is recommended that further monitoring and management action be undertaken to ensure the permanence of this important and historically ignored species in the area.

CONTENTS

FIGURES

TABLES

1

Chapter One: General Introduction

1.1. LIFE HISTORY OF CHONDRICHTHYAN FISHES

Sharks and their relatives (class Chondrichthyes) evolved in marine ecosystems over 400 million years (Grogan et al., 2004). It is a diverse group found in all the world's oceans, where they play an important role in the function of marine communities as predators and scavengers. Although shark landings represent a small portion (1%) of global fish landings (Dulvy et al., 2008), catches have exceeded the recruitment ability of several populations that are now classified as depleted or at risk (Walker, 1998, Baum et al., 2003, Ferretti et al., 2013). A few notable examples of this are the school shark (*Galeorhinus galeus*) (Olsen, 1959, Punt and Walker, 1998, Flood et al., 2012), basking shark (*Cetorhinus maximus*) (Parker and Stott, 1965), all sawfish (genus *Pristis*) and several other species in locations like the Mediterranean (Ferretti et al., 2013), the Gulf of Mexico (Bonfil, 1997) and the North Atlantic (Baum et al., 2003).

As the global concern for the status of chondrichthyan fishes has risen, investment in research to inform management has also increased (Au et al., 2008). Informed management is particularly important for these species, which are at a higher risk of overexploitation due to several factors:

I. Conservative life histories (i.e. slow growth, low fecundity, long lives and low natural mortality) make chondrichthyans susceptible to over-exploitation and slow to recover from depletion (Smith et al., 1998).

II. Many species utilise coastal nurseries and are susceptible to coastal fisheries and environmental degradation caused by anthropogenic activity and changing climactic conditions (Heupel et al., 2007).

III. Many species face unsustainable fishing pressure from targeted and incidental capture (Bensley et al., 2009). Often chondrichthyans are not the primary target but are captured as bycatch (i.e. discarded, although handling and injuries may affect them adversely or result in post-release mortalities), or by-product (i.e. not targeted but retained). In these fisheries, management focus tends to be on the target spp., often at the expense of less productive, non-target populations (Walker, 1998). Although good progress has been made in management, catches of many chondrichthyan species remain unregulated (Camhi et al., 2009, Dulvy et al., 2014).

IV. Important aspects of the life history, biology and spatial ecology of several populations remain poorly understood, resulting in a general uncertainty in management (Worm, et al., 2013).

In Australia, sharks represent an important component in multiple high value fisheries such as the western tuna and billfish fishery, the northern prawn fishery, the Coral Sea fishery (including bycatch in trawling) and most significantly the southern and eastern scalefish and shark fishery (Simpfendorfer and Donohue, 1998). Current management is largely based on the Australian second national plan of action for the conservation of sharks (AFMA) (Shiffman et al., 2012). The plan strives for management of all chondrichthyan species to be informed by local research on their life history and biology (Walker et al., 2005, 2007, 2008). However, that does not mean that Australian shark populations are impervious from overexploitation because important information gaps still exist, particularly for species with low commercial value.

1.1.1. Importance of life-history traits

The life history of an organism can be broadly defined as the strategies adopted through each of the aspects of its life cycle, including early development, habitat utilisation, feeding ecology, maturation, reproductive periodicity and fecundity as well as growth and longevity (Caughley, 1977). Chondrichthyan fishes tend to display conservative, k-selected strategies resulting in low productivity, low tolerance for exploitation, and long recovery times (García et al., 2008). While this may be a reasonable generalization, there is a wide range of life-history strategies within the class (Cortés, 2000). For example, Pacific Spiny Dogfish (*Squalus suckleyi*) in the North East Pacific produce an average of 6-7 pups every second year (Ketchen, 1975), mature as late as 35 years of age and live up to 107 years (Tribuzio et al., 2012). In contrast, the Australian sharp-nose shark (*Rizoprionodon taylori)* matures after one year, gives birth to up to 10 young annually and lives for up to ~seven years (Waltrick et al., 2012).

The life history of a species is dictated by its evolutionary history and can be influenced by both environmental (e.g. system productivity) and ecological factors (e.g. interactions with other species, density dependent processes) (Musick, 1999). There is also variability between the individuals within a population; therefore, life-history traits can change dynamically through time (e.g. Taylor et al., 2009), which highlights the importance of using timely and locally derived data to inform management.

1.1.2. Age and growth

Reliable age estimation and growth data are crucial components of stock assessments for many species (Cailliet et al., 2006) and facilitate the derivation of productivity and mortality rates (Frisk et al., 2001). Accordingly, a miscalculation of age could have severe repercussions in the management of a fishery. For example, one of the measurements used in the management of gummy sharks (*Mustelus antarticus*) are size limits, which are intended to allow a couple of years post maturity before individuals enter the fishery. However, incorrect age estimates were

shown to produce an underestimation of age at maturity that could result in higher than desired catch rates of individuals that had not yet reproduced (Officer et al., 1996).

Establishing the age of long-lived species can be challenging and is traditionally achieved using calcifying structures with accretionary growth (Cailliet et al., 2006). When growth varies at predictable intervals (e.g. seasonally), an alternating pattern of clear and opaque bands is formed in calcified structures and, if validated as forming at well-defined periodic intervals, can be used, in conjunction with the date of capture and age of formation of the first growth zone, to estimate age. In studies of chondrichthyan fishes, vertebrae are most commonly used (White et al., 2014), although dorsal spines (Holden and Meadows, 1962), neural arches (McFarlane et al., 2002) and caudal thorns (Cailliet et al., 2006) have also been used. Obtaining accurate age estimates can be difficult in species with poor calcification and although histological staining or decalcification can be used to enhance growth bands (Correia and Figueiredo, 1997), validated age determination remains elusive for many species.

One of the main assumptions of ageing is that growth increments are deposited periodically and at a consistent rate through the individual's life (Musick and Bonfil, 2005). However, rates of calcium deposition are affected by the environment and may vary between species or even populations of the same species (Simpfendorfer, 2005). Accordingly, it is important that age estimation studies validate their results. However, absolute age validation is rarely achievable as it can be logistically challenging, time consuming, and expensive (Branstetter, 1987). A variety of techniques can be used to achieve age verification or partial validation, these include chemical marking of the hard parts of wild animals that are tagged and released (Campana, 2001), bomb carbon dating (Campana et al., 2006), length increment analysis (Natanson et al., 2002), edge analysis (Natanson and Cailliet, 1990) and captive experiments (Branstetter, 1987). These techniques require relatively large samples representative of all age classes, therefore, age

validation of chondrichthyans remains a technical challenge and the best approach is usually a combination of the above-mentioned techniques.

1.1.3. Reproduction and maturity

All chondrichthyans have internal fertilization and relatively low fecundity (Au et al., 2008), however, the group has evolved a variety of reproductive strategies (Conrath and Musick, 2012). These can be broadly categorised into lecithotrophic strategies (i.e. oviparity, yolk sac viviparity and ovoviparity) in which there is no maternal input during embryonic development, and matrotrophic strategies (i.e. placental, histotrophic, oophagous and intra-uterine cannibalism) in which there is maternal input during gestation (Carrier et al., 2004). Many species also display complex adaptations like long-term sperm storage (Conrath and Musick, 2012), intermittent embryonic development (Waltrick et al., 2012) and even parthenogenesis (Chapman et al., 2007). Therefore, a thorough understanding of a species' reproductive biology is an essential input into the analysis of demographics, providing reliable estimates of fecundity, age at maturity, temporality of the reproductive cycle and gestation length.

Maturity in chondrichthyans is frequently sexually dimorphic and can be highly variable between individuals (Carrier et al., 2004). Male sharks are considered mature when claspers are fully calcified and rotary (Conrath and Musick, 2012), and when gonads produce mature sperm (Musick and Bonfil, 2005). In field studies it is common practice to determine the maturity of individuals based solely on clasper calcification. However, for species in which gonads and claspers do not develop concurrently, consideration of clasper status alone can underestimate size at maturity (Pratt Jr, 2001). Thus, in species where these relationships are unknown, maturity should not be estimated using claspers alone.

Female sharks are considered mature when they have a fully developed reproductive tract and can successfully produce offspring (Otake, 1990). Most commonly, assessment of female maturity is achieved through direct examination of the ovaries and uterus, requiring lethal

sampling (e.g. Avsar, 2001, Bubley et al., 2013, Cigdem Yigin and Ismen, 2013, Hanchet, 1988). By sampling seasonally, it is possible to determine gestation length, temporal patterns in the reproductive cycle, and fecundity (Ketchen, 1972). Although direct examination remains the most reliable approach (Heupel and Simpfendorfer, 2010), lethal sampling may not always be possible or desirable (e.g. endangered species). Non-lethal sampling methods used in the past include visual examination of the hymen (Bruce, 1992), blood biochemistry to determine variation in hormonal concentrations (e.g. Awruch et al., 2008) and ultrasound imaging (Sulikowski et al., 2016).

1.1.4. Mortality

Survivorship is the result of both natural mortality (M) and fishing mortality (F). Direct estimation of mortality rates in the field requires detailed and extensive fisheries dependent catch data or an intense tag-recapture regime (Heupel and Simpfendorfer, 2011). More recently, acoustic telemetry has been used to estimate natural mortality of juvenile Blacktip Sharks *(Carcharhinus limbatus*) in a coastal nursery (Heupel and Simpfendorfer, 2002). However, direct estimation of natural mortality remains unobtainable for most species and methods that permit mortality to be estimated indirectly are increasingly popular (Simpfendorfer et al., 2005). Indirect methods build on life-history theory to provide estimates of mortality using associated life-history invariants (Hoenig and Gruber, 1990, Chen and Watanabe, 1989) and include age dependent and age independent methods.

Age independent methods (e.g. Pauly, 1980, Hoenig, 1983, Jensen, 1996) assume that natural mortality is identical across all age classes (Simpfendorfer et al., 2005). Expanding on those concepts, age dependent methods (e.g. Peterson and Wroblewski, 1984, Chen and Watanabe, 1989) produce a U-shaped curve with increased mortality of young and very old cohorts. This approach is likely to be more biological meaningful and is consistent with mortality patterns estimated directly in the field (Heupel and Simpfendorfer, 2002). Importantly, most of the

commonly used models for mortality inference in chondrichthyans tend to be derived from other taxa (mostly teleost fish) (i.e. Simpfendorfer, 1999). Therefore, when inferred mortality estimates are used for stock assessment or demographic analysis, it is important to test the sensitivity of the model to a range of mortality values (McAuley et al., 2007) and if possible, use catch curves for comparison.

1.1.5. Application of life-history data for fisheries management

Population dynamics are determined by the life-history traits of a species, and therefore influenced by demographic and environmental processes (Frisk et al., 2001). Generalized approaches that model these dynamics can provide estimates of net productivity and the intrinsic growth rate of a population. Unlike quantitative stock assessments, demographic analyses only require basic life-history information making them an attractive tool for the management of data-poor species (Frisk et al., 2001, Cortés, 2002). Consequently, demographic analysis has become an increasingly popular tool in the study of chondrichthyans (Simpfendorfer, 2005).

Demographic analysis traditionally combines retrospective and prospective techniques, providing insights into the current status of a population (i.e. how historic factors have influenced the demography of the species) and the potential effect of future variability in biological parameters and/or fishing pressure on the population (i.e. Carlson and Baremore, 2003, Cortés, 1999, Cortés, 1995, Cortés and Parsons, 1996). Further, in the absence of detailed historic fisheries-dependent data, useful reference points for stock assessment can be derived analytically using demographic models (Brooks et al., 2010). While demographic models cannot generate realistic predictions of stock size without additional data, they provide reference points that allow managers to make inferences about productivity and compare different populations and species (Smith et al., 1998) rendering them a powerful management tool.

1.2. STUDY SPECIES: SPINY DOGFISH (*SQUALUS ACANTHIAS*)

The spiny dogfish (*Squalus acanthias*) is an abundant demersal shark belonging to the Squalidae family (Fordham et al., 2006). The species is cosmopolitan, with subpopulations in the North Atlantic, Mediterranean Sea, Black Sea, South Atlantic and South Pacific, including an Australasian subpopulation (Veríssimo et al., 2010). The North Pacific spiny dogfish population was recently re-classified as a separate species, *S. suckleyi*, based on morphological and genetic differences (Ebert et al., 2010). The present study is focused on *S. acanthias* in the Australasia region; specifically, in western Tasmania.

Squalus acanthias can live up to 52 years and typically reach lengths of ~100 cm, although individuals as large as 140 cm have been reported in the North Atlantic (Soldat, 1982). Age at maturity is locally variable, but usually occurs at around 11 years of age (Yigin et al., 2013 and references therein). Females are lecithotrophic viviparous and gestation lasts 22-24 months, one of the longest among chondrichthyans (Jones and Geen, 1977b). They are generalist feeders, preying opportunistically on small invertebrates and fish (Alonso et al., 2002). These life-history characteristics are some of the most conservative reported in chondrichthyans and mean the species has very low productivity and long generation times (Cortés, 2002). Therefore, despite their abundance they are particularly vulnerable to overexploitation and slow to recover.

Squalus acanthias are a highly mobile species with vast spatial distributions across continental shelf areas. They are primarily demersal and can be found in depths of up to 900 m, although they usually remain above 200 m (Fordham et al., 2006). They are typically highly migratory, undertaking seasonal movements across large areas (several 100 km) (Carlson et al., 2014). These migratory movements have been linked with the reproductive cycle and thermal preferences (7–15 °C) (Thorburn et al., 2015). Furthermore, size and sexual segregation is common (Ketchen, 1975). Due to the species' aggregating and segregating behaviours, it is difficult to establish stock status using traditional fishing metrics based on catch per unit effort

(Haugen et al., 2017) and groups of high demographic importance (e.g. mature females) can be at a higher risk of overexploitation (Fordham, 2005).

In the Northern Hemisphere, fisheries for *Squalus* sp. are documented as early as the 1870s when they were targeted as a source of oil for lighting and machinery lubrication (Beamish et al., 2009). They were later targeted as a part of the post-world war II liver oil fishery for vitamin A, as a source of oil for cattle and in government subsidized eradication fisheries (Beamish et al., 2009). Intense fishing pressure has resulted in severe biomass declines and ongoing over-exploitation of multiple populations, particularly in the Northern Hemisphere. For example, in the Northeast Atlantic, Black Sea and Northwest Atlantic, declines from baseline biomass levels of 95%, 60% and 75% respectively have been reported (Fordham, 2005). In response the International Union for the Conservation of Nature (IUCN) has classified the species as 'vulnerable' across its range and subpopulations in the Mediterranean and North Atlantic as 'endangered' and 'critically endangered' respectively (Fordham et al., 2006).

Largely because of their historical and current commercial importance *S. acanthias* is one of the best studied chondrichthyan species. Furthermore, they are regularly used as a model species for long lived, wide ranging chondrichthyans due to their abundance and small size (e.g. Jones and Geen, 1977a, Campana et al., 2006). Surprisingly, although the general biology of the species is known, their ecologic role and significance remains poorly understood (Fordham, 2005). Given that *S. acanthias* are long lived and have complex spatial strategies (migratory behaviour and spatial sexual segregation), population specific knowledge of movement and life-history patterns is essential to inform management. However, the majority of what is known about *S. acanthias* comes from populations in the Northern Hemisphere, and significant data gaps exist across the species' southern range.

1.2.1. Squalus acanthias in Australia

Squalus acanthias occur throughout the coastal and shelf waters of southern Australia, where the species is caught as bycatch in several fisheries (Walker et al., 2005, Williams and Schaap, 1992). Their distributional range overlaps the Southern and Eastern Scalefish and Shark Fishery, one of the largest commercial fisheries in Australia (Walker et al., 2007), but as there is no commercial demand for the species catches are usually discarded. However, due to their conservative life history, potentially high post-release mortality rates (Braccini et al., 2012), and lack of local research, they are considered to be at high risk of overexploitation (Walker et al., 2007). Based on these factors, the species was assigned a residual risk ranking of medium in the most recent assessment of shark species for the national plan of action (Walker et al., 2008), but no specific conservation actions have been implemented (Bensley et al., 2009).

The species is thought to be particularly abundant in coastal waters off Tasmania (Last and Stevens, 2009), although their distribution and movements patterns are largely unknown. The species has been reported in various Tasmanian estuaries and large coastal embayments (Williams and Schaap, 1992, Edgar, 1991) and it has been suggested that breeding occurs in these areas (Last and Stevens, 2009). Published evidence in support of this suggestion is lacking and the existence of inshore breeding grounds requires further exploration. In Tasmania there are a number of areas that have been declared as Shark Refuge Areas (SRAs) where the targeting or retention of most elasmobranch species, including *S. acanthias* is prohibited (DPIPWE, 2013). However, the species has been shown to be particularly susceptible to capture in gillnets, likely because of their schooling habit and their spines, which readily become entangled in the meshes (Lyle et al., 2014). In the most recent ecological risk assessment for the Tasmanian general graball and non-reef gillnet sub-fisheries, *S. acanthias* was classified as a medium and high-risk species, respectively (Lyle et al., 2014).

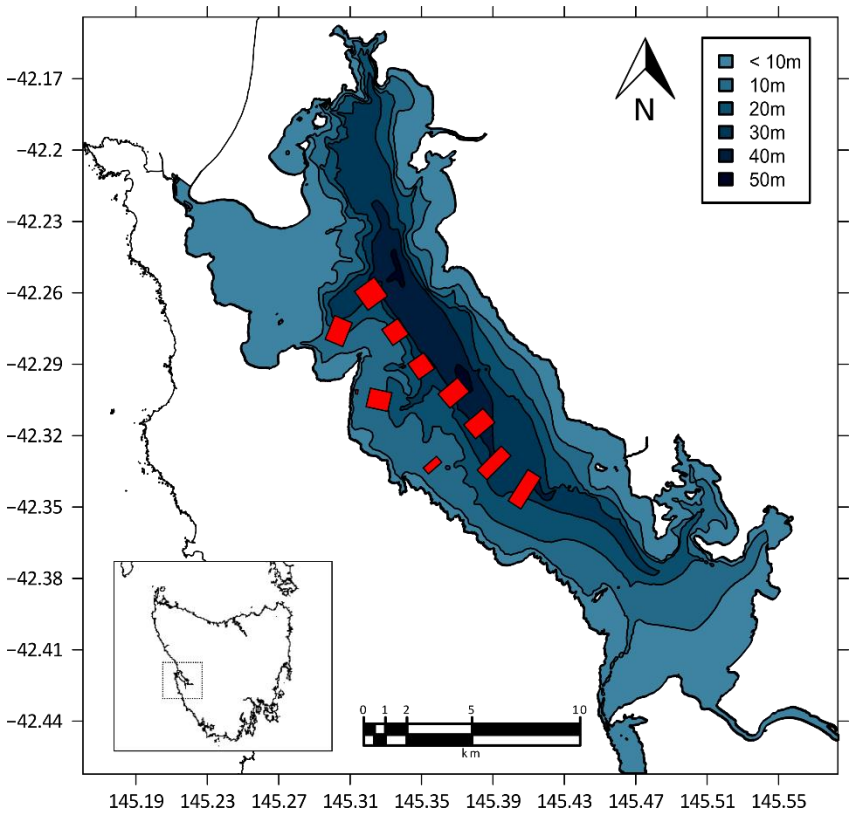

Figure 1.1. Map and bathymetric profile of Macquarie Harbour, Tasmania. Red polygons represent the salmonid aquaculture leases where the sea-pens are moored.

1.3. STUDY SITE: MACQUARIE HARBOUR, WESTERN TASMANIA

Macquarie Harbour is an estuarine system located in the west coast of Tasmania (42.3198° S, 145.3843° E) where *S. acanthias* is locally abundant (Lyle et al 2014). Macquarie Harbour was likely formed as a glacial valley during the Pleistocene, which flooded after the last glacial maxima (Macphail et al., 1993). The harbour is relatively shallow, with a maximum depth of ~50 m in the central basin. It has a surface area of approximately 276 km^2 and connects with the Southern Ocean via a shallow (<10 m) and narrow entrance channel. This feature greatly restricts water exchange with the ocean, resulting in a relatively stagnant deep-water mass that is naturally low in dissolved oxygen (DO) (Cresswell et al., 1989).

The aforementioned characteristics affect the hydrology of Macquarie Harbour, causing the separation of the water column into three distinct layers (Cresswell et al., 1989). A freshwater layer near the surface of the harbour is the result of high discharge from the Gordon River

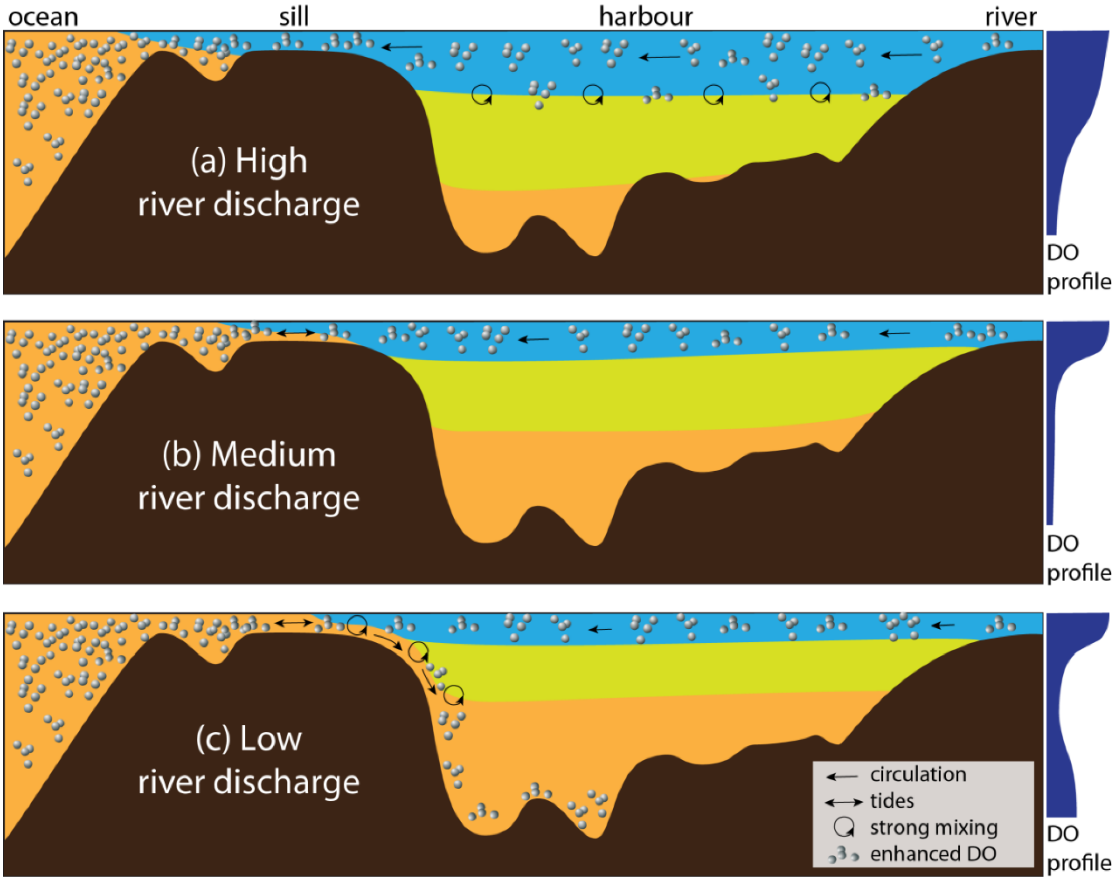

Figure 1.2. Schematic representation of stratification in the water column within Macquarie Harbour, as well as the physical processes affecting oxygen exchange under variable river discharge conditions. Layers in the water column represent oceanic water (orange), brackish water (yellow) and riverine or freshwater (blue). Diagram reproduced from Ross and McLeod (2017).

catchment. Water from the river has a dark coloration caused by tannins, which leach from the heavily forested areas upstream (Carpenter et al., 1991). This limits light penetration and acts as a thermal barrier, creating a unique deep-sea like environment in Macquarie Harbour. A second layer comprised of brackish water is located below the halocline and has naturally low DO. When saline, dense, oceanic water overflows into the harbour it enters below the upper two layers forming the third layer with physico-chemistry typical of oceanic waters. The stratification of the water column creates a vertical gradient in the physico-chemical profile of the harbour, affecting ecologically important factors like temperature, salinity and dissolved oxygen (Figure 1.2) (Ross and MacLeod, 2017). For example, although temperature is seasonally variable near the surface, conditions become more stable at increasing depths, with only small temperature fluctuations past 20 m (Carpenter et al., 1991).

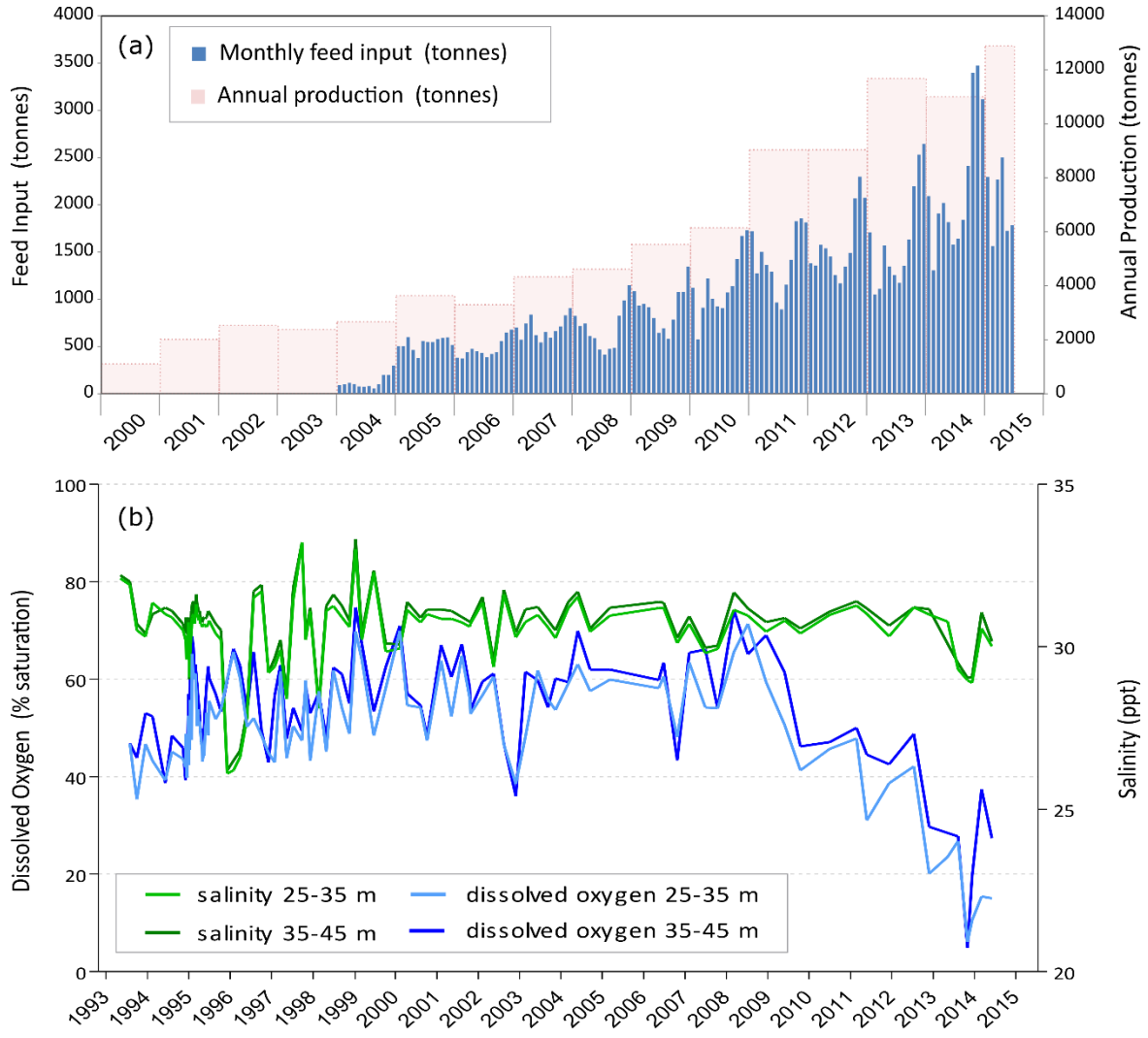

Figure 1.3. Top: annual aquaculture biomass production and feed input levels in Macquarie Harbour from 2000 to 2015. **Bottom:** dissolved oxygen levels at two depths in Macquarie Harbour from 1993 to 2015. Figures reproduced from Ross and McLeod (2017)

Conditions in Macquarie Harbour are naturally depauperate and native fauna is mostly comprised of small infauna (Edgar et al., 1999). The silty bottom and low light penetration allow several typically deep-water species to live in the shallow harbour, including *S. acanthias* (Richardson et al., 1997). Vertebrate fauna mainly comprise small teleost and elasmobranch fishes, although large animals like cetaceans and pinnipeds sometimes enter the harbour for short periods (Driessen and Mallick, 2003). Macquarie Harbour and Bathurst Harbour (~150 km south of Macquarie Harbour) are the only two known habitats of the endangered Maugean skate (*Zearaja maugeana*), although the species has not been recorded in the latter for over 20 years and may now be locally extinct (Treloar et al., 2017). *Squalus acanthias* are also common

in Macquarie Harbour and have been reported in high abundances throughout the harbour for much of the year (Lyle et al., 2014). Although the species distribution and seasonal use of Macquarie Harbour are presently unknown, their abundance suggests they are likely to have an important ecological role in the system as predators. Despite its remote location, Macquarie Harbour is a popular tourism destination and has been used in various industries like aquaculture, logging and mining. Many of the rivers in the region were historically used in mine dewatering (i.e. removal of ground water from a mine), dispersing contaminated water and acidic mine drainage downstream into Macquarie Harbour. Although this practice has been stopped, sediments in the harbour still have elevated concentrations of heavy metals like zinc and copper (Carpenter et al., 1991). The stratified water column in Macquarie Harbour is ideal for the culture of salmonids as the freshwater negates the need for anti-amoebic gill disease baths, prompting the development of a large aquaculture operation for Atlantic salmon (*Salmo salar*) and to a lesser extent rainbow trout (*Oncorhynchus mykiss*). Commercial farming operations began in the late 1980's and have grown steadily since, with annual production exceeding 12,000 tonnes in recent years (Figure 1.3a) (Ross and MacLeod, 2017). There is a recreational gillnet fishery in Macquarie Harbour for which the target is predominantly flounder (*Rhombosolea tapirine* and *Ammotretis rostratus*) and escaped salmonids (Lyle et al. 2014). *Squalus acanthias* are taken as a bycatch of this fishery and although rarely retained are known to experience moderately high post-capture mortality rates (Lyle et al. 2014).

A growing body of recent evidence has demonstrated environmental degradation in Macquarie Harbour, raising concerns for the health of the ecosystem. Most significantly, there is an ongoing downwards trend in DO levels beginning in 2009, which coincides with the major expansion in salmonid aquaculture in the harbour (Figure 1.3b). Aquaculture contributes to the organic loading in the system through fish excretion and spillover food pellets. The bacterial breakdown of this waste has been estimated to be responsible on average for 3-12% of benthic biological oxygen demand (BOD) in Macquarie Harbour (Ross et al., 2016). When the BOD

exceeds the supply of oxygen from physical processes, DO levels decrease (Middelburg et al., 2005).

In Macquarie Harbour replenishment of DO is likely supplied mostly by river discharge and episodic deep-water recharge events in which oxygen-rich oceanic water overflows into the system. Oxygen in surface water is incorporated into the system through vertical mixing which can be inhibited by increased stratification or enhanced by high winds or strong currents. Therefore, replenishment of DO in Macquarie Harbour is naturally low and is likely to be strongly affected by changes in river flow (Figure 1.2). These conditions limit the system's ability to recover from current low DO conditions, which have already resulted in mass mortality of infauna communities in close proximity to the marine farm leases (Ross et al., 2016) and will likely have further direct impacts on the ecology of the harbour.

The physical properties responsible for Macquarie Harbour's unique ecosystem make it particularly vulnerable to environmental disturbances. The expansion of aquaculture production in the harbour has likely contributed to the observed declines in water quality and environmental degradation of the area. Despite this, management action is hampered by a lack of historical data and poor understanding of the biogeochemical processes in the harbour. In response, a large multidisciplinary research effort has emerged in recent years with the aim to improve knowledge about the system and the impact of past and ongoing environmental changes on the local ecology and native fauna.

The occurrence of *S. acanthias* in semi-enclosed coastal habitats is well documented and has been linked with seasonal migrations motivated by their reproductive cycle (i.e. breeding) or environmental preferences (Thorburn et al., 2015). It is likely that *S. acanthias* use Macquarie Harbour in a similar way; however, it is unknown how the life history of the population may be affected by the unique environment in the estuary, which represents an unusual habitat for the species. Therefore, locally apposite knowledge of their biology and ecology is necessary to

understand how the species may be affected by past and ongoing environmental impacts in the harbour. For example, low DO levels can affect the distribution of a species or their prey and may even disrupt development and physiological processes. This could have important consequences for *S. acanthias,* a species whose life history makes them vulnerable to depletion, in particular if Macquarie Harbour is a nursery area or environmental refuge for the population.

In the months preceding this study, a plan specifying a twofold increase of production biomass in Macquarie Harbour aquaculture was approved (DPIPWE, 2012). The actual impact that commercial fish farming has had on the local fauna cannot be determined due to a lack of data preceding the onset of aquaculture. Therefore, local research on the biology and ecology of key species may help establish a baseline for impact assessment prior to the implementation of the proposed changes.

1.4. OBJECTIVES AND THESIS STRUCTURE

It is widely accepted that the life history and movement ecology of wild species are linked to the environment they experience; and therefore, changes in environmental conditions can be expected to affect these traits (García et al., 2008). However, the effect that extreme conditions or rapid environmental changes may have on long-lived organisms, such as sharks, are still poorly understood (Taylor et al., 2009). Given the overlap between environmentally impactful human activity (e.g. pollution, fisheries, aquaculture) and marine ecosystems, it will be important for the appropriate management of species living in these areas, to improve our understanding of such interactions and how they may influence biological traits in these populations. An effective way to accomplish this goal, is by contrasting the known ecologic and biological traits of well understood model-species and populations exposed to unusual environmental conditions or impacted habitats.

Reflecting on this, the primary goal of this work was to investigate the potential effects that a degraded environment may have on the biology and ecology of long-lived elasmobranch

populations, contributing to the understanding of the plasticity of these processes and how they may be influenced by environmental and anthropogenic pressures (e.g. aquaculture). This was accomplished through the study of *S. acanthias* in Macquarie Harbour, placing the biological characteristics of this population in the context of *S. acanthias* populations elsewhere. Furthermore, due to the growing concern for the health of Macquarie Harbour and the need for informed managerial decisions regarding the interactions between *S. acanthias* and aquaculture, this study endeavoured to provide apposite information to help improve understand the local life history and ecology of the species. To accomplish these goals, the study focused on three main objectives:

1) determine the spatial and temporal patterns of movement of adult *S. acanthias* in Macquarie Harbour (Chapter 2);
2) determine key life-history and ecologic traits of the species in the area (i.e. reproduction, diet (Chapter 3), age and growth (Chapter 4)); and
3) apply the newly acquired knowledge of the population's life-history traits to construct a demographic model of that can be used to inform on the status of the population and identify the need for management intervention (Chapter 5).

The findings of this study are presented as standalone chapters prepared as manuscripts for publication.

Chapter 2 describes seasonal sex-specific patterns of movement, distribution and social behaviour of *S. acanthias* within Macquarie Harbour. Knowledge of when and how each sex uses Macquarie Harbour can help define the importance of the system for the spatial ecology of the species. In turn, this can be used to predict the vulnerability of the species to past and ongoing environmental degradation in Macquarie Harbour. To accomplish this, passive acoustic telemetry was used to track tagged individuals over a 1.5-year period.

Chapter 3 presents an assessment of the reproductive biology and diet of *S. acanthias* in Macquarie Harbour. This included observations on the seasonality of reproduction and gestation, as well as fecundity and the size at which maturity is reached in the population. Diet was explored using a combination of traditional (stomach content analysis) and modern (stable isotope analysis) techniques. Knowledge of these processes provides important information about how the species uses Macquarie Harbour, and is an essential component of demographic analysis.

Chapter 4 details age and growth of *S. acanthias* in Macquarie Harbour. This chapter provides the first estimates of age and growth for the species in Australian waters and discusses how these estimates compare with other populations. Dorsal spines have been traditionally used to age this species; however, their reliability has recently been brought into question. Here, spine derived age estimates are compared to those obtained from vertebrae, which are not prone to wearing and breakage.

Chapter 5 acts as a general discussion of the overall findings from this thesis. A new set of models and simulations are introduced to provide a contextual framework to integrate and interpret the broad implications of this work. Specifically, data acquired in the previous three chapters were used to conduct a demographic analysis of *S. acanthias* in Macquarie Harbour. The potential effects of environmental disturbances in the area were explored through prospective simulations, providing a powerful toolset for the management of the species.

This thesis provides statistical tools to aid in demographic analyses, life-history investigation and movement modelling of *S. acanthias* in Macquarie Harbour; bringing it together in an overarching narrative that represents the first in-depth study of the species in Australian waters and the Southern Hemisphere. These data address questions about the population status and dynamics of the species, offering new avenues of inquiry and providing tools for improved

management. The results of this work will be relevant for the study and conservation of. *S. acanthias* through its range, as well as other ecologically important long-lived species.

2

Chapter Two: Spatial Dynamics, Movement and Behaviourally-Mediated Space Utilization

2.1. INTRODUCTION

Life-history traits are strongly related to population dynamics, making them a common focus of management (Hutchings, 2002). Life histories are influenced by the energy resources available to an individual, this in turn is dependent on the environment through the complex interaction of ecological (e.g. primary productivity, diversity, biological interactions) and abiotic factors (e.g. physical structure, temperature, salinity, etc) (García et al., 2008). These factors can vary temporally and result in large (i.e. migratory or ontogenetic) and small (i.e. diel) scale movements, changing the individual's spatial distribution (Carlson et al., 2014). Therefore, it is important to understand the strategies and behaviours that cause changes to a species spatial distribution, as knowledge of space use and site affiliations can help identify important habitats and how they are utilised, a crucial aspect of spatial management. Habitat selection and movement are driven by trade-offs between optimal foraging opportunities, physiological preferences, biological interactions and reproduction (Sagarese et al., 2014b). Therefore, changes in spatial-ecology, as well as the motivators driving movement and site selection, can play a crucial role in dictating population dynamics of a species (Sims, 2003).

Squalus acanthias is a small demersal shark found in temperate waters circumglobally (Fordham et al., 2006). Although the life-history traits of the species are variable between populations, they are, in general, late maturing and long lived, with low fecundity and a gestation period of

~24 months (Hisaw and Albert, 1947, Fordham et al., 2006). Their conservative life history, susceptibility to fishing gear (Lyle et al., 2014, McCully et al., 2013) and aggregating behaviour (Holden, 1965), make the species highly vulnerable to overexploitation and large biomass declines have occurred as a result of fishing (Taylor et al., 2009), prompting the IUCN to classify the species as vulnerable and populations in the NE Atlantic as critically endangered (Fordham, 2006). Notably, individual populations are distributed across vast geographic ranges and undertake large-scale seasonal migrations (Holden, 1965, Templeman, 1976, Thorburn et al., 2015), meaning that locally derived knowledge on the spatial ecology of a population is necessary to inform spatial management strategies.

Knowledge of the spatial ecology of *S. acanthias* in Australia is limited to a basic understanding of distribution based on capture locations (Last and Stevens, 2009) and, apart from some regulations relevant to elasmobranchs in general, there are no species-specific management arrangements in place for the species (Walker et al., 2007). *Squalus acanthias* are highly mobile and considered migratory, with individuals undertaking trans-Atlantic movement (Templeman, 1976). However, a growing number of studies have shown that some populations of *S. acanthias* and *S. suckleyi* do not undertake large-scale movements and instead, they remain localized within large coastal areas where seasonal migrations still occur, but at scales of 100s rather than 1000s of kilometres (Carlson et al., 2014, McFarlane and King, 2003). Coastal habitats act as important foraging areas for many elasmobranchs (Munroe et al., 2014a), offer protection from predation to certain life stages (e.g. nurseries) (Heupel et al., 2007) and/or provide access to preferred environmental conditions (e.g. acting as thermal refuges) (Thorburn et al., 2015). Coastal habitats tend to have a high level of exposure to anthropogenic disturbances and therefore, coastal populations may have a higher susceptibility to localized declines (Chin et al., 2010). Adult *S. acanthias* typically segregate into subgroups based on sex, each with distinct spatial distributions (Sims, 2005). Sex specific patterns of movement in the species have been linked to environmental factors, such as thermal preference (Sagarese et al., 2014b, Shepherd et

al., 2002), and to biological processes, such as their reproductive cycle (Hanchet, 1988). Recently it has been shown that subgroups in coastal populations (e.g. pre-reproductive females) can have a variety of spatial utilisation strategies, suggesting that certain environments may promote residential behaviour (Thorburn et al., 2015). Thus, in addition to understanding patterns of space use, knowledge of the behavioural drivers of movement is necessary to understand the spatial ecology of individual populations.

Macquarie Harbour is a coastal estuary in western Tasmania, where, based on fisheries independent sampling, adult *S. acanthias* of both sexes are known to occur throughout the year (Lyle et al., 2014). The harbour is mostly shallow, although deeper sites can be found in the central basin (0–55 m). There is a large influx of freshwater from surrounding catchments, resulting in a strongly stratified vertical profile, with the halocline occurring at depths of between 2-15 m (Cresswell et al., 1989). *Squalus acanthias* are typically stenohaline (Fordham et al., 2006 and references therein); therefore, Macquarie Harbour represents an unusual habitat for the species. The system is connected to the ocean by a narrow and shallow entrance channel (~10 m deep), possibly restricting animal movements in and out of the system by creating a physical (i.e. shallow and narrow passage) and environmental barrier (i.e. salinity). The species' distribution, movement and connectivity to the open ocean are unknown and it is unclear whether individuals in Macquarie Harbour represent a spatially isolated population or are part of a larger Australian population.

Macquarie Harbour has been impacted by historic mining runoff (i.e. high heavy metal input and acidification) and more recently by significant nutrient inputs associated with a large and expanding salmonid aquaculture industry. Nutrients in cultured fish excrement and spillover feed pellets are likely to result in increased bacterial oxygen consumption. Severely hypoxic benthic conditions and significant declines in DO throughout the water column are now commonly reported in Macquarie Harbour (Ross and MacLeod, 2017), rising concerns about

the degradation of environmental conditions in the area. These impacts could result in physiological stress for species such as *S. acanthias* or force individuals to relocate, as well as impacting the abundance and diversity of benthic species that they prey on (Islam, 2005). Gaining a local understanding of the patterns of broad-scale movement (migratory movement) and space use of *S. acanthias* will help better understand the importance of Macquarie Harbour for the species as well as the potential impact that recent environmental changes may have on the population.

A commonly used tool in the study of movement of marine species is passive acoustic telemetry. This technique requires two main components, a transmitter tag attached to, or inserted in, the animal and listening station(s) that record when the tag is within detection range (Heupel et al., 2005). Through the strategic placement of the receivers, it is possible to explore a variety of aspects of a species spatial ecology, including habitat selection, temporal space usage and

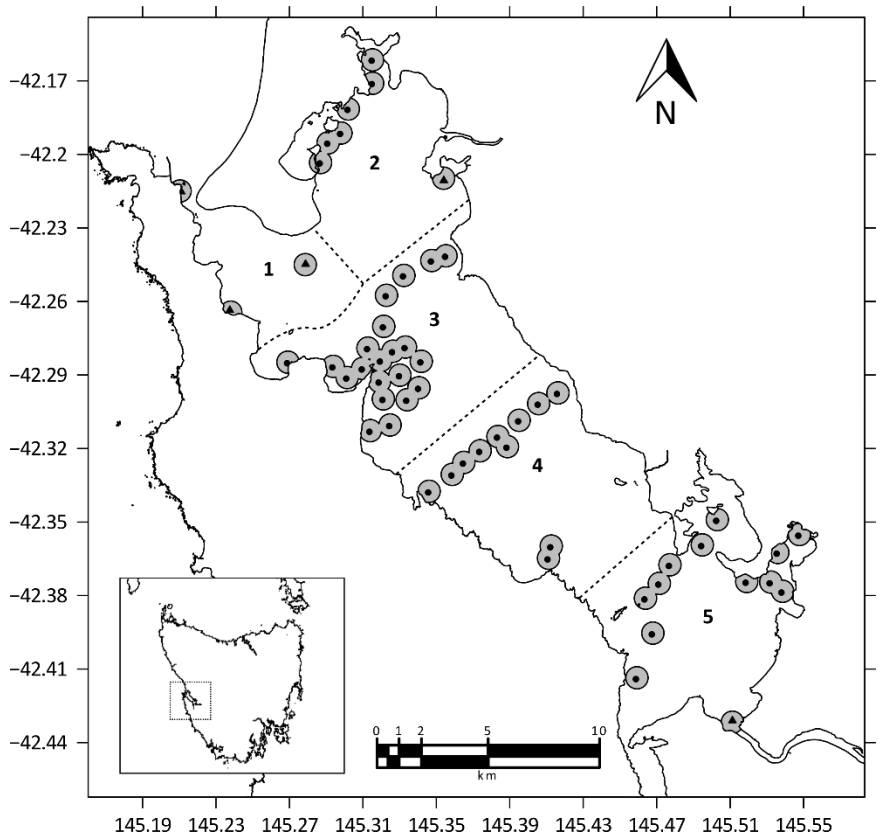

Figure 2.1. Study site at Macquarie Harbour, Tasmania. Location of the acoustic receivers is marked by solid circles and triangles (gate receivers). Average detection range of the receivers (Bell et al., 2016) is represented by the grey polygons. The study area was divided into five focus areas: 1) harbour mouth, 2) lower basin, 3) central basin, 4) middle receiver curtain and 5) upper basin.

migration patterns (Heupel et al., 2006). However, the analysis of this type of data tends to be restricted to descriptive, location-based methods (Heupel et al., 2004). Looking to build a more reliable quantitative approach, a number of improved modelling methods have been developed in recent times; these include state transition models (Jacoby et al., 2012a, Stehfest et al., 2015), random walk movement models (Brownian and Levy flight) (Pagès et al., 2013, Papastamatiou et al., 2013) and social network analysis (Jacoby et al., 2012b, Stehfest et al., 2013). These new approaches each have specific advantages over simple descriptive or semi-quantitative approaches and can explore different aspects of the same data set, providing information on space use, behaviour or sociality. While most researchers choose to implement one type of model, by combining several of these techniques into a multi-model framework, it may be possible to obtain a complementary and broader interpretation of the available data combining spatial, temporal and behavioural analyses.

In this chapter, findings are presented for a long-term passive-tracking study focusing on adult *S. acanthias* tagged in Macquarie Harbour, Tasmania. A multi-model analytical framework is used to integrate spatial, temporal and behavioural components derived from random walk, state-transition and neural network analysis. The primary aim of this work is to determine *S. acanthias'* broad-scale movements in and out of Macquarie Harbour, site association patterns within the system, including overlap with aquaculture operations, and whether these patterns are influenced by sex and thus related to the reproductive cycle.

2.2. MATERIALS AND METHODS

In October 2013, fifty-three VR2W-69 kHz acoustic receivers (VEMCO Ltd, Halifax, Canada) were deployed within Macquarie Harbour (42.3198° S, 145.3843° E; Figure 2.1) as part of a multi-species tracking project (Bell et al., 2016). The listening stations were deployed at depths ranging from 5 to 50 m. Most receivers were suspended in the water column by being attached to an anchor weight, securing them to the substrate, and a sub-surface buoy, ensuring the

omnidirectional hydrophone was consistently oriented upright. Eight receivers at sites with depths >20 m were fitted with a remote acoustic release to aid with retrieval and four others were attached to fixed structures such as aquaculture infrastructure, piers and navigational markers. The array had an irregular design (Figure 2.1) consisting of: Three units deployed at the entrance of the harbour and two at the mouth of the main tributaries, forming detection gates to record possible movement of tagged individuals in and out of the system. To monitor longitudinal movement within the estuary, 37 receivers were positioned at four parallel locations bisecting the harbour transversely (i.e. curtains of receivers with partially overlapping detection ranges) and at potential interest locations (i.e. the inlet in the south east side of the upper basin). Finally, 12 receivers arranged as an irregular grid were deployed around the western shore of the central basin to examine finer-scale movement behaviour within the vicinity of aquaculture operations.

To account for spatiotemporal variation in detection ranges (i.e. the average maximum distance at which a transmitter will be successfully detected), the performance of the array was tested by Bell et al., (2016) using a multi-tiered range-testing regime based on the recommendations by Kessel et al. (2014). Receiver performance and detection range variability was monitored throughout the full observation period both passively (i.e. fixed sentinel tags deployed at a fixed distance of 6 receivers throughout the study) as well as actively (i.e. recurrent drift tests every 3 months). Receivers had an estimated mean detection range of 405 m across the array and performance was not variable through time. A detailed description of the range testing methodology and results can be found in Bell et al. (2016).

2.2.1. Acoustic tagging

Squalus acanthias were captured using bottom set long lines consisting of 50 baited hooks placed at 5 m intervals on a 250 m lead core rope. All individuals were measured (total length, as measured from the snout to the extended upper lobe of the caudal fin measured to the nearest

mm), sexed and a uniquely labelled rototag was attached to the first dorsal fin to enable identification in case of accidental recapture. Coded acoustic transmitters (VEMCO V13-1H; 13 mm diameter; 6 g weight in water; transmitting at: 69 kHz with 60-120 sec random delay; predicted battery life of 513 days) were surgically implanted in 12 males (480-600 mm total length (TL)) and 10 females (724-875 mm TL) during two sampling trips to Macquarie Harbour, twenty in November 2013 and two in February 2014. Tagging was restricted to adults to help explore possible migratory patterns linked to the reproductive cycle of the population, animals were deemed to be adults based on the reported size at maturity for the species (Chapter 3). Individuals were inverted to induce tonic immobility and placed in a V-shaped piece of foam. A small incision (1–2 cm) was made anterior to the cloaca on the abdominal wall and the transmitter inserted into the peritoneal cavity. The incision was closed using surgical sutures (Braun Safil® HS26s), after which animals were immediately returned to the water and held next to the boat until able to swim unassisted. Aseptic techniques were used throughout.

Tagged individuals were released near the receiver closest to their capture location so that the tags operational status could be verified. Three females and six males appeared to have died more or less immediately following release (tags were detected constantly throughout the study period by the release receiver). The reason for these mortalities in unknown, although all occurred during a single tagging event, suggesting that holding and/or environmental conditions were suboptimal. The handling procedure was unlikely to be the issue, as it was used successfully prior to and following this incident. The remaining individuals remained alive through the duration of this study and are the basis for all subsequent analysis.

2.2.2. Data analysis

In March 2015 all receivers were recovered, detection records were downloaded and compiled. Data following tagging release were monitored to determine when individuals returned to normal behaviour and it was decided to remove the first two days of data from all individuals.

Signal collision may result in false positives, so data were filtered to retain only instances where at least 3 detections occurred within an hour (Heupel et al., 2005). Only two instances were found that did not fit this condition which were excluded from further analyses. A detailed description of data preparation and model implementation can be found in Appendix 1.

A variable number of detections throughout the day has been linked to diel changes in behaviour (Payne et al., 2010), therefore alternating stages of high and low activity would be expected to result in a matching shift in detectability. As such, diel patterns in detection frequency were explored by using one-way ANOVA. Data were pooled for all individuals and divided by hour of the day; the data conformed to the assumptions of ANOVA and required no further transformation.

2.2.3. Residency and migration

Seasonal use of Macquarie Harbour by *S. acanthias* was estimated using a standard residency index (RI), calculated by dividing the number of days an individual was successfully detected in the study area by the total number of days in the observation period and multiplying the result by 100 (as per March et al., 2010). Given that the acoustic array did not provide full spatial coverage, it was possible for individuals to be within Macquarie Harbour but remain undetected, particularly in areas with lower receiver density like the upper harbour. Periods of non-detection were not necessarily indicative of movement outside the system; therefore, an individual was considered to have left the harbour only if they were detected at one of the entrance gate receivers prior to a period of absence (exit) or prior to reappearance (re-entry). The effect of sampling season and sex on RI were tested using a two-way ANOVA, with the data requiring no transformation.

2.2.4. Seasonal home-ranges

To reduce location bias introduced by having fixed receivers, short-term centres of activity (COA) were calculated (Simpfendorfer et al., 2002). This is accomplished by using an algorithm

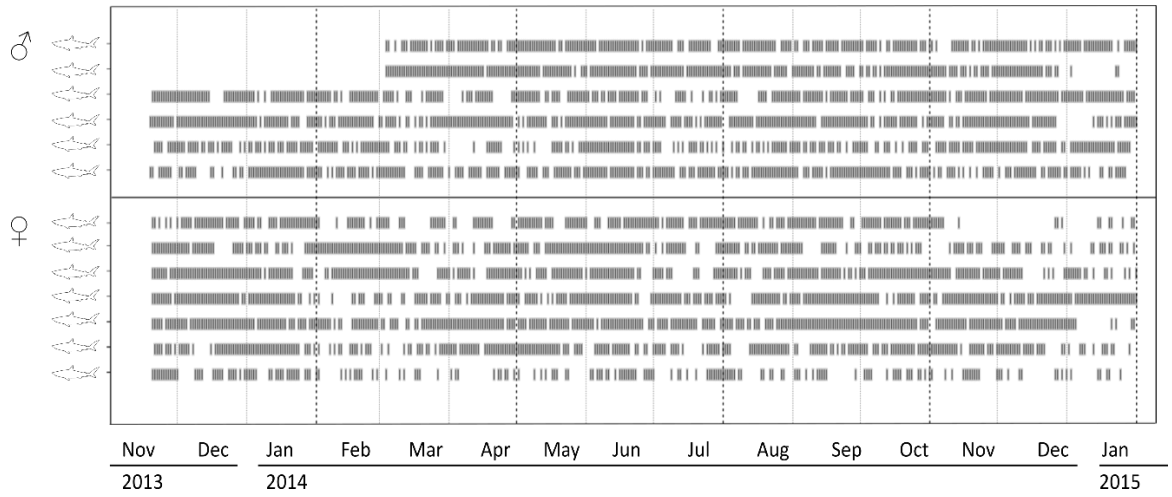

Figure 2.2. Daily detection summary for tagged *S. acanthias* in Macquarie Harbour for the time between Nov 2013 and Feb 2015. Marks (l) indicate a positive daily identification within the array.

to calculate mean locations for each individual, weighted by the number of detections recorded by different receivers over a set time interval (Δ_t). A Δ_t of 30 minutes was considered adequate based on a visual examination of the mobility of the species, receiver density and average detection frequency. The resulting data were used as the basis for all subsequent home range calculations.

Utilization density distributions (UD) were calculated foe each individual using a Brownian-Bridge Movement Model (BBMM) and used to determine the 50 % isopleth (core home range or c50%), 95% isopleth (extended home range or c95 %) and 99% (total activity area or c99 %) (as per Pages et al., 2007). To explore seasonality the analysis was broken down into five 3-month periods, roughly coinciding with austral seasons; "spring 2013" (Nov 2013–Jan 2014), "summer 2014" (Feb 2014–Apr 2014), "autumn 2014" (May 2014–Jul 2014), "winter 2014" (Aug 2014–Oct 2014) and "spring 2014" (Nov 2014–Jan 2015). BBMMs incorporate detection error into the UD calculation by assuming that every detection is not a constant fixed location but rather a Gaussian probability function around that point (Horne et al., 2007). Detection error in this study was assumed to be equivalent to the mean radius at 50% detection accuracy for the array; a value of 405 m was used based on range testing results, as no spatiotemporal

variation in detection ranges was found. This method considers the dependency between subsequent detections by modelling the movement between each pair of successful detections as a random walk described by Brownian motion (Horne et al., 2007). Instances when the time lag between subsequent detections exceeded 1260 minutes were excluded in order to avoid the over inflation of probabilities. This value was calculated as the approximate time it would take an average individual to swim between the two most distant receivers in adjacent curtains (~20 km) at the reported average swimming speed of the species (~0.27 m s^{-1}) (Domenici et al., 2004).

Calculations were performed using R statistical software (R Core Team, 2013), based on the code in the BBMM package (Nielson et al., 2011). Probabilities were estimated using a regular grid matrix representing the study area, where an individual cell size of 10 m^2 was chosen as the base unit. The cells in the matrix that overlapped with land were treated as an impassable barrier to avoid any probability being assigned to those areas. To assess sex-based differences in space use, UDs were aggregated by sex and re-scaled to a total value of 1. The home range isopleths were then recalculated to represent sex-specific home ranges. The extent of the area in km^2 represented by the UD polygons (i.e. home range isopleths) was used to calculate the proportion of the UDs that overlapped with the aquaculture leases. Extended home range area use by depth was calculated in 10 m intervals and scaled by the total available area of each depth bin in the harbour to reflect relative importance and depth affiliation patterns for each sex.

2.2.5. Site affinity and movement behaviour

Movement behaviour in relation to site affinity was analysed using state-space network analysis (Jacoby et al., 2012a). Receivers were grouped into five focus regions based on geographical proximity and design function (e.g. receiver curtains) (Figure 2.1) with each representing a behavioural state in the model. Movement was assumed to be a Markovian process and transition probability matrices was calculated using a discrete stationary Markov-Chain model. The steady state of the resulting transition matrices was calculated (i.e. the leading eigenvector

of the transpose transition matrix) as an estimate of the relative importance of the different areas (i.e. centrality) (Jacoby et al., 2012a).

The transition matrices were calculated using SOCPROG 2.6 (Whitehead, 2009), which undergoes an optimization process using the Poisson approximation to maximize the likelihood of the identification data. This approach does not account for temporal shifts in behaviour, so season specific analyses (using the seasons described earlier) were conducted to account for possible seasonal differences in movement behaviour (Stehfest et al., 2014). A Mantel test (Schnell et al., 1985) was used to determine the relatedness of the transition matrices between the sexes. A non-detection state was not included in the calculations due to the lack of evidence for movement outside the harbour (detailed below). To prevent the modelling of unrealistic movement, the transition probability between non-adjacent areas was fixed to zero a-priori.

2.2.6. Seasonal patterns of sociality

Social networks were used to analyse the associations between individuals throughout the study period. Data were analysed using SOCPROG 2.6 (Whitehead, 2009) software to calculate associations based on the observed connections between pairs of individuals (dyads). To account for temporal changes in social behaviour, the dataset was subdivided into seasons as per the UD calculations. A sampling interval (SI) of 4 hours was chosen to test for associations where the grouping factors were the individual receivers in the array. As none of the tagged individuals were deemed to leave the study area (see Results Section 2.3.1), a simple association index was chosen as an adequate metric of sociality (time a dyad was associated / total time both individuals were identified in the sampling interval) (Jacoby et al., 2012b). Network statistics (i.e. social differentiation index, gregariousness) were also calculated for each sex and a Mantel's test was used to assess the difference in association strength between sexes as a grouping variable. Preferred/avoided associations were tested using a Monte Carlo permutation

Table 2.1 Extent of male and female core (50%) and home (95%) ranges based on BBMM utilization distributions per season. Metrics for the core and extended ranges include the total area in km2, the area of the UD that overlaps with the aquaculture leases and the proportion of the total area of Macquarie Harbour covered by the UD.

		Core home range (c50)			Extended home range (c95)		
		Area (km^2)	Lease overlap (km^2)	Available (%)	Area (km^2)	Lease overlap (km^2)	Available (%)
Males							
Spring	2013-2014	15.1	2.4	5.3	135.7	8.2	47.4
Summer	2014	5.5	1.8	1.9	123.1	2.2	43.0
Autumn	2014	33.7	2.8	11.8	200.6	9.2	70.1
Winter	2014	36.9	2.3	12.9	189.6	9.3	66.3
Spring	2014-2015	18.9	2.3	6.6	161.9	7.5	56.6
Females							
Spring	2013-2014	10.6	1.5	3.7	67.5	5.9	23.6
Summer	2014	4.2	0.8	1.5	80.1	5.0	28.0
Autumn	2014	22.9	2.5	8.0	139.8	9.3	48.9
Winter	2014	17.4	2.3	6.1	109.2	8.1	38.2
Spring	2014-2015	7.1	1.1	2.5	64.0	5.8	22.4

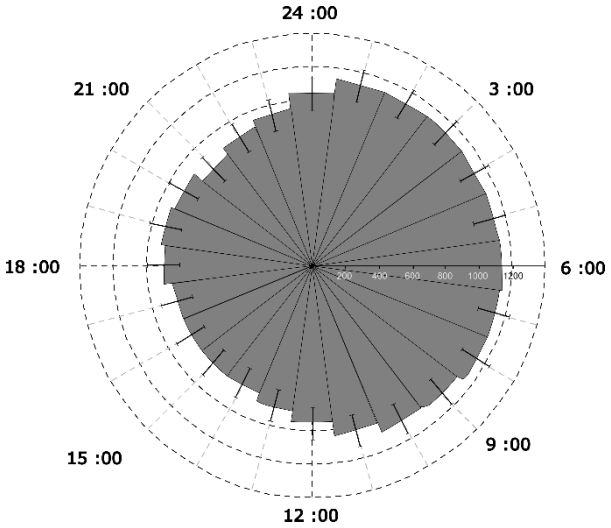

Figure 2.3. Chronograph of mean detection number (± S.E.) by hour of the day for all individuals throughout the complete observation period.

test for associations within samples, which controls for differences in gregariousness (Whitehead, 2009).

Two methods were used to investigate social grouping patterns: 1) a network diagram was generated using non-metric multidimensional scaling, where stress was used to determine the usefulness of the diagram (Whitehead, 2009); and 2) hierarchical agglomerative cluster analysis was conducted to assess structure based on associative strength between individuals as a

measurement of distance. Clusters are prone to defining connections even when they are not significant, so the maximum modularity (i.e. index of cluster structure and association strength) of the system was calculated to determine a cut-off point for grouping (Whitehead, 2009). Modularity was calculated from permutations, as it provides a more conservative estimate that accounts for gregariousness (Whitehead, 2009). This is important as network analysis by default does not consider the causality of associations, and high gregariousness can lead to the identification of false associations (Jacoby et al., 2012a).

To explore short term temporal dynamics in sociality, the lagged association rates (LARs) were calculated (as per Stehfest et al., 2013). This analysis determines the probability that any two individuals remain associated through time regardless of their past associative state (i.e. a Markovian process). Mean LAR was plotted against time using a moving average window to reduce noise. Additionally, sex specific LARs were plotted, along with the overall null association rate (NAR). The NAR represents the probability of any two individuals becoming associated independently of previous associations, and thus constitutes the cut-off point for preferential association.

2.3. RESULTS

From November 2013 to February 2015 a total of 311,192 unique detections were recorded, with records for individual sharks ranging from 7,200 to 41,418 (mean ± SD of 23,239 ± 2,740). There was no significant difference in total detections by sex ($t = -0.084$, $df = 10$, $p = 0.934$). Time of day did not significantly affect number of detections ($F=3.424$, $p=0.065$), suggesting any patterns in diel activity did not affect detectability. A summary of tagged Individuals is provided in Appendix 1.

Table 2.2. Residency index (RI) by season based on proportion of daily detections from total days in observation period. Summary indicates mean RI by sex and pooled. NAs indicate periods prior to tagging where no data were available.

		Males							Total
		SD02	SD04	SD05	SD06	SD31	SD33		♂
Spring	2013-2014	69.3	75	90.5	78.4	NA	NA		78.3
Summer	2014	75.3	53.9	81.8	55.7	98.3	81.4		74.4
Autumn	2014	82.6	69.6	82.6	59.8	90.1	85.9		78.4
Winter	2014	89	64.8	87.8	69.2	84.8	82.6		79.7
Spring	2014-2015	58.1	83.9	65.2	93.4	63.1	83.7		74.6
		Females							Total
		SD11	SD12	SD13	SD14	SD15	SD16	SD20	♀
Spring	2013-2014	64.3	74.7	93.1	87.8	91.8	77	78.4	81
Summer	2014	31.5	62.9	79.8	56.8	75.6	71.9	42.1	60.1
Autumn	2014	47.8	69.6	85.9	77.2	69.6	79.4	78.3	72.5
Winter	2014	41.3	69.2	92.3	75	85.9	64	81.5	72.8
Spring	2014-2015	30.6	68.5	70.8	87.8	62	51.2	16.5	55.3

2.3.1. Residency and migration

Detectability was high for all individuals throughout the study period (Table 2.2), although there were occasional short periods of non-detection that ranged from one day to two weeks (Figure 2.2). As no individual was detected by any of the three most proximate receivers to the harbour entrance it is highly unlikely that any of the tagged individuals departed the estuary during these periods of absence and each was subsequently detected inside the harbour (Figure 2.2). The RI of females and males differed significantly ($F=4.922$, $p=0.03$), but sampling season had no effect ($F=2.167$, $p=0.146$). The combined detection range of all receivers in the array (based on a detection range (radial) of 405 m) accounted for ~26 km^2 within the harbour (~9% of surface area) and the mean daily detectability for the duration of the study was 74.75% (± 7.46 SD).

2.3.2. Space-use and movement

The BBMM model was able to successfully account for areas of known use where no receivers were present (i.e. where *S. acanthias* were captured on longlines during sampling), revealing continuous movement within and among different areas of the harbour (Figure 2.4a, Figure

2.5a). Based on the UD's, the core home range of the sampled individuals was consistently located in the deep central basin of the harbour (Figure 2.4, Figure 2.5). The extended range of both sexes shifted seasonally, with both using a smaller area of the harbour during summer (Table 2.1). Males underwent a synchronous change in site use during winter, when their distribution expanded into the upper basin of the harbour (Figure 2.4a). This area was exclusively used by male *S. acanthias* and includes a portion of the harbour designated a world heritage area (WHA). However, a large proportion of their range was outside of the WHA, suggesting continuous movement in and out of the reserve.

The transition matrices calculated using the Markov-Chain model resulted in patterns inconsistent with random movement (Figure 2.4b, Figure 2.5b). These results complement the UDs and add behavioural information, such as the frequency and direction of transitions made by the sharks between adjacent areas. Site affinity and directionality of movement were similar through the year for females (Figure 2.5b) whereas male movement patterns showed that the centrality of area 5 (upper basin) grew during winter but area 3 (central basin) remained important, suggesting that individuals moved continuously between the two areas (Figure 2.4b).

The area covered by the aquaculture leases was found to directly overlap with the home ranges of both males and females (Table 2.2). Habitats with depths >30m accounted for an average of 53.37% (± 4.06% SD) of the total area of the extended home ranges. However, when scaled by availability (based on surface area), the relative importance of these deeper habitats (>30m) increased to 75.58% (±5.68% SD) (Figure 2.6).

2.3.1. Social interactions

Values for the index of social differentiation varied between seasons, with the first four seasons indicating well-defined social structures (> 0.5). Spring 2014-2015 had a higher value (~2.0), which is indicative of extreme differentiation and weak relationships (Table 2.3). The strength

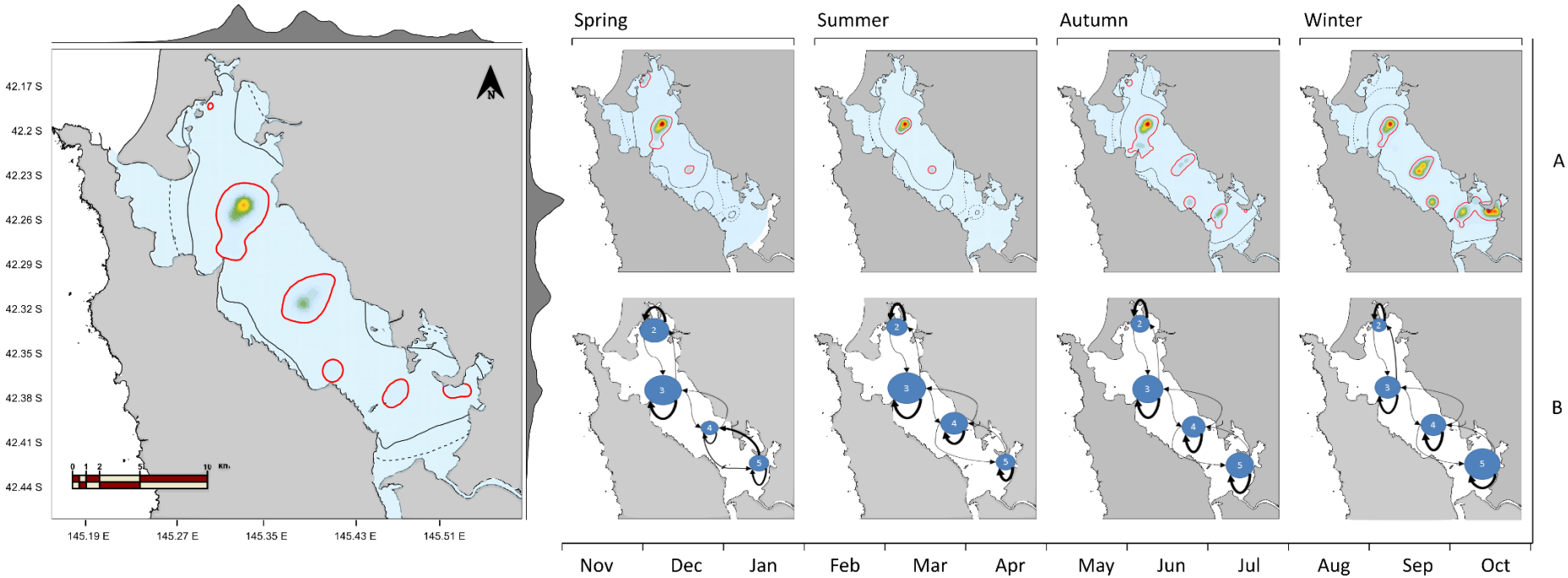

Figure 2.4. Space use of male *S. acanthias* in Macquarie Harbour. Main plot represents the summary of the full study period. **(A)** BBMM UDs by season with core home range c50 (—) , extended home range c95 (—) and total activity area c99 (---) isopleths. **(B)** Network diagram for the state-transition model, where node diameter (solid circles) represents the relative importance (eigenvector centrality) of the different areas (numbered from 1-5), edges (arrows) represent movement between the different areas weighted (line thickness) by the discrete transition probability in the Markov Chain transition matrix.

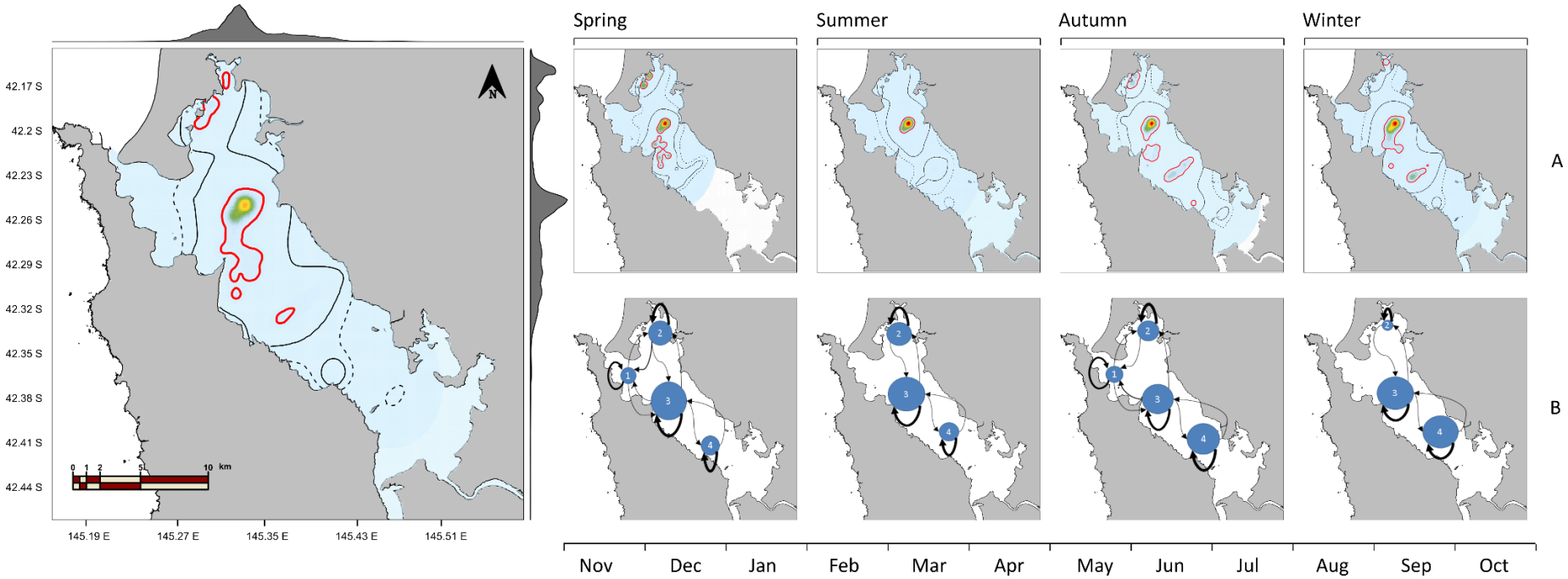

Figure 2.5. Space use of female *S. acanthias* in Macquarie Harbour. Main plot represents the summary of the full study period. **(A)** BBMM UDs by season with core home range c50 (—) , extended home range c95 (—) and total activity area c99 (---) isopleths. **(B)** Network diagram for the state-transition model, where node diameter (solid circles) represents the relative importance (eigenvector centrality) of the different areas (numbered from 1-5), edges (arrows) represent movement between the different areas weighted (line thickness) by the discrete transition probability in the Markov Chain transition matrix.

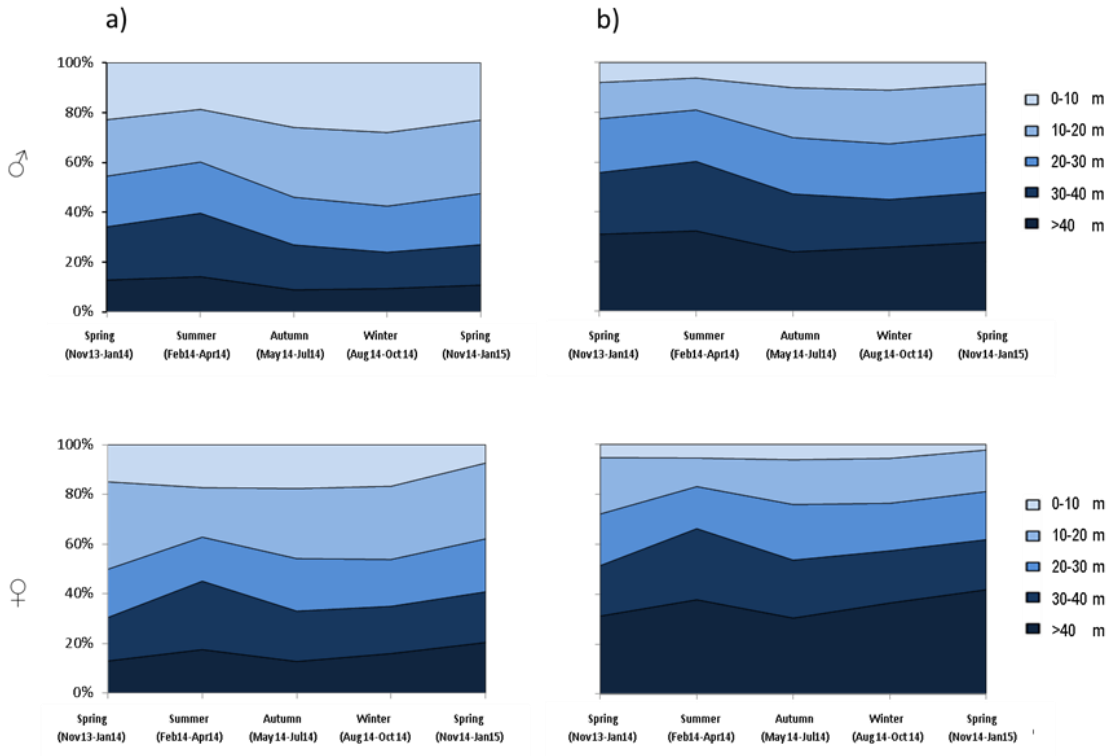

Figure 2.6. **(a)** Depth preference based on total area of extended home ranges (c95) for *S. acanthias*. **(b)** Depth preference weighted by total availability of depth in Macquarie Harbour.

of association (gregariousness) varied seasonally, with the highest associative index occurring in summer. However, the Mantel's matrix correlation test found no significant difference between sexes for any of the individual seasons ($p>0.001$). Cluster analysis identified distinct groupings in each of the seasons (Figure 2.7), however, in the first four seasons the modularity of the optimal arrangement was <0.3, suggesting that those divisions had marginal use for group identification (Whitehead, 2009). Conversely, during spring 2014-2015, optimal modularity was 0.369, supporting the validity of the community division indicated in the cluster diagram (Appendix 1).

The coefficient of variation for preferred associations and non-zero elements was significantly higher than expected from random behaviour, indicating that the population displayed both preferred and avoided associations (Table 2.3). Preferred and avoided associations were significantly different when permuting between sexes, indicating a sex specific difference in the way that associations are formed. This indicates that both males and females are equally

Table 2. 3. Summary network statistics based on sociality for all dyads presented by season. Sampling period (sp) show the number of sampling units (4 hr) per season where associations were recorded. Indices of gregariousness and mean group size per sampling period are presented for pooled and sex specific data (± s.e.).

	Spring 2013-2014			Summer 2014			Autumn 2014			Winter 2014			Spring 2014-2015		
	All	M	F	All	M	F	All	M	F	All	M	F	All	M	F
Sampling periods (sp)	445			536			557			555			523		
Mean ids/ sp	173.4			118.52			130.38			97.89			72.05		
Proportion of individuals /sp	45.92			31.43			35.63			34.65			27.15		
Mean associations / dyad	18.62			33.18			12.86			7.73			5.49		
Social differentiation index	0.702			0.818			0.593			0.919			1.754		
Gregariousness	0.20 (0.08)	0.24 (0.1)	0.18 (0.6)	0.54 (0.1)	0.56 (0.11)	0.53 (0.11)	0.16 (0.06)	0.19(0.04)	0.19(0.08)	0.13 (0.06)	0.1 (0.03)	0.15(0.07)	0.16 (0.07)	0.16 (0.04)	0.16 (0.1)
Group size	3.04 (0.81)	3.45 (1.03)	2.81 (0.62)	7.54 (1.26)	7.68 (1.29)	7.41 (1.32)	3.25 (0.75)	3.26 (0.52)	3.24 (0.94)	2.25 (0.68)	2.2 (0.34)	2.78 (0.8)	2.9 (0.9)	2.89 (0.48)	2.9 (1.19)

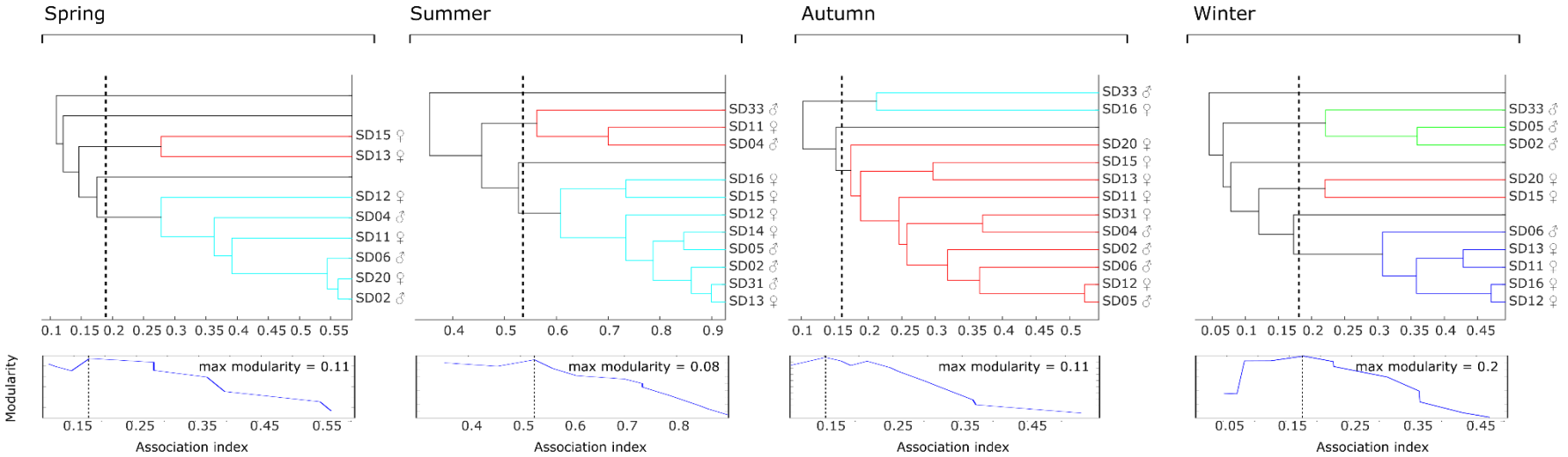

Figure 2.7. Dendrograms of social groups by austral season based on network analysis using a simple association index (i.e. both individuals in a dyad identified). The cut off point for clustering (---) is determined from the maximum modularity as calculated from gregariousness.

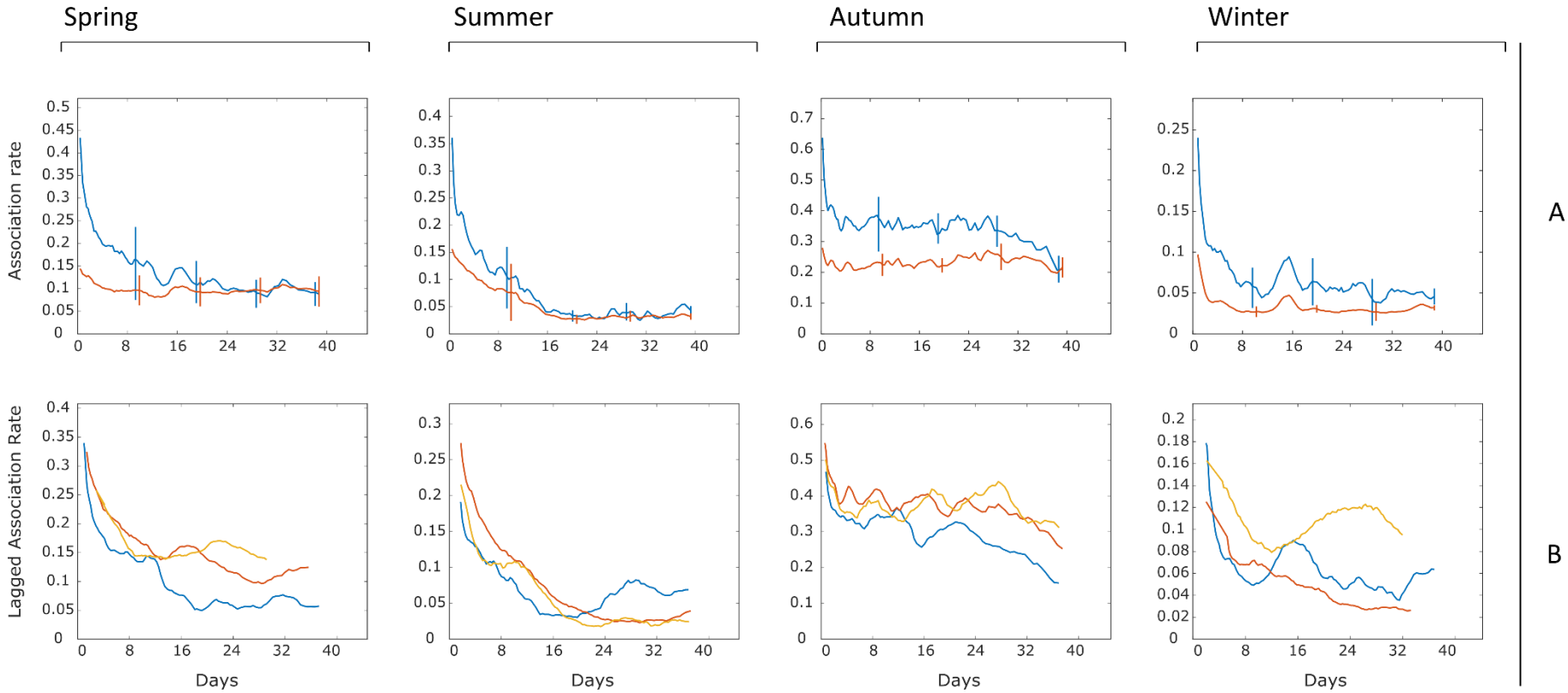

Figure 2.8. (A) Temporal patterns of sociality for the different seasons, showing lagged-association rates (—) and null association rates (—) with variance calculated using jack-knife resampling (500 reps). **(B)** Lagged-association rates between groups, male to male (—), female to female (—) and female to male (—).

expected to form associations throughout the year, but the strength of those associations varies seasonally.

The analysis of lag association rates for all seasons resulted in a rapidly decaying model which continued to decrease until it intersected with the null association rate at ~8 days (Figure 2.8). After that point any previous interactions no longer influenced the probability of the individuals in the dyad reassociating. The joint interpretation of these patterns suggest that the species does group at higher rates than would be expected at random; however preferred associations are not long term and therefore the identification of distinct community divisions or social groups was inconclusive.

2.4. DISCUSSION

During the 15-month observation period none of the tagged individuals left the system suggesting long-term residency, a finding that was unexpected when considered in the context of typical behaviour for the species (i.e. migratory movement resulting in seasonally variable distributions). Both sexes shared a similar core home-range around the central basin of the harbour and unlike other populations, males and females did not show fully segregated spatial ranges at any time, presumably due to the small size of Macquarie Harbour. There was, however, evidence for an expansion of the extended home range of males during winter, which resulted in differences in sex-specific movement behaviour and patterns of sociality. These observations differ markedly from what is known about the species elsewhere and are suggestive of long-term residential behaviour that is likely an adaption to the unique environment of Macquarie Harbour.

Tracking studies typically attempt to characterize individual behaviour to generalise on population level patterns (Bartumeus et al., 2013). The design of location-based movement experiments and subsequent data analysis are ultimately determined by the research objectives and species-specific behaviours of the target species. In the present study a multi-model data

analysis framework was implemented using state transition models, BBMMs and social network analysis. Each of these synthesize different aspects of the dataset (e.g. location, temporality, gaps between subsequent detections, etc.) and therefore can be used to describe distinct aspects of the special ecology of the study species. In combination, these models showed the areas being used by the population (UD), which sites are used more frequently (home-range), how individuals move between different sites (transition matrix), how social groups are structured (social-network), and how those patterns change depending through time. Thus, this analytic framework is likely to be a valuable tool for the study of space use and movement of coastal species, as it was shown to facilitate a more robust interpretation of detection data over any of the individual models alone.

The high degree of site fidelity exhibited by *S. acanthias* in Macquarie Harbour differs from previous reports of space utilization and dispersal in the species or the closely related *S. suckleyi*. *Squalus acanthias* were traditionally thought to be highly migratory over vast geographic scales (Templeman, 1976) but as more is discovered about the species, it is becoming evident that there is a high degree of intraspecific variability in the scales of movement patterns between populations (Holden, 1965, Carlson et al., 2014). Coastal populations in some regions fragment into transient and resident components (Carlson et al., 2014, McFarlane and King, 2003), with the former undertaking large scale oceanic migrations, and the latter remaining more localized within large coastal areas, including semi enclosed water bodies such as bays, straits and lochs (Thorburn et al., 2015). Although resident populations have a smaller spatial extent than migratory ones, they still display localized migratory behaviour (Sagarese et al., 2014, Shepherd et al., 2002). For example, *S. acanthias* in the Celtic Sea have been shown to undergo annual latitudinal migrations (i.e. north-south) (Thorburn et al., 2015). By contrast, the population in Macquarie Harbour remained resident within the estuary, a maximum area of 275 km^2, with no evidence of migratory movement of tagged adults outside the harbour.

Macquarie Harbour is connected to the ocean by a shallow and narrow channel with only small quantities of marine water entering the estuary during flood tides and fresh/brackish waters exiting on ebb tide (Cresswell et al., 1989). Since adult *S. acanthias* are mostly stenohaline, this shallow corridor could act as a physical and/or environmental barrier that limits their connectivity with the coastal waters outside of the estuary. For instance, it is unlikely that individuals could travel from the central basin to the ocean without encountering fresh/brackish waters. This is supported by the fact that no individuals were detected by the receivers at the harbour entrance (Figure 2.1). If exchange outside of the harbour is extremely limited, the population may be effectively isolated, implying that the entire life cycle is conducted within the harbour. The use of partially isolated water bodies has been observed in other elasmobranch species such as the sawfish (*Pristis perotteti*) of Lake Nicaragua. This anadromous species traditionally gives birth in freshwater or estuarine systems, where juveniles spend their early life before migrating to oceanic waters, however, in Lake Nicaragua active tracking showed that *P. perotteti* can conduct their entire life cycle inside the large freshwater lake (Thorson, 1982). It may be possible that these semi-enclosed water bodies offer benefits (i.e. access to food, predator protection, increased juvenile survivorship) that encourage philopatry in species that would otherwise display migratory behaviours.

The mechanisms underlying dispersal and migratory behaviour in *S. acanthias* are thought to be mainly driven by thermal preferences and their reproductive cycle (Carlson et al., 2014). A study of *S. acanthias* in Loch Etive, Scotland showed that two non-breeding females over-wintered in the system, remaining resident within the local area for 10 months while other individuals moved offshore (Thorburn et al., 2015). It has been suggested that semi-enclosed coastal sites may act as thermal refuges, promoting residential behaviour in subcomponents of a larger population (Shepherd et al., 2002, Thorburn et al., 2015). However, in Macquarie Harbour adults of both sexes were detected within the system more or less continuously for over 15 months. Assuming the typical two-year reproductive cycle in the species, this suggests

that all stages of the reproductive cycle occurred within the system for all individuals tagged, suggesting that long-term residency could be prevalent in the whole population and not just a subcomponent. Biological sampling to assess the reproductive condition of the population undertaken concurrently to this study supports this conclusion (Chapter 3), strengthening the evidence to suggest that *S. acanthias* in Macquarie Harbour may constitute an isolated population.

Due to the lack of historical data on the spatial dynamics of this population, it is unclear if the behaviour represents a long-term adaptation, or a short-term response to prevailing environmental conditions, such as a temporary shift in migratory patterns driven by resource availability, noting the recent dependence on aquaculture overfeed as a food source (Chapter 3). Regardless, these patterns are likely to have important implications for the population in the future. The species is a highly successful generalist that inhabits a wide range of habitats circumglobally (Fordham et al., 2006). Theory dictates that low levels of habitat specialisation result in increased fitness and resilience to environmental change (Munroe et al., 2014b), allowing a species to easily alter their spatial distribution as an adaptive mechanism to cope with demographic or environmental pressures. A restriction in range, as seen in Macquarie Harbour, could, however, increase the population's vulnerability to habitat disruptions and exacerbate the negative effects of density dependent processes.

1.1.1. Seasonal patterns of distribution, movement and behaviour within Macquarie Harbour

The location and extent of the core range of all tagged individuals was persistent throughout the year, suggesting that the central basin of Macquarie Harbour plays a crucial role in the spatial ecology of the population. In contrast, the extended home ranges of all individuals expanded during winter and areas in the upper basin of the harbour were used exclusively by males. Elsewhere, males and females of the species are known to segregate spatially (Dell'Apa et al., 2014, Sagarese et al., 2014a), however, due to the restricted extent of Macquarie Harbour,

full spatial partitioning may not be possible. This is supported by the persistent overlap in most of the distribution of males and females, particularly at the core of their ranges; suggesting that despite small differences in extended ranges, males and females in Macquarie Harbour do not have spatially segregated ranges. Furthermore, sex-specific patterns of association and avoidance revealed that during winter, males and females are less likely to associate or to remain associated with individuals of the opposite sex. Therefore, the patterns of sociality seen in this study may represent an alternative mechanism for segregation based on changes in associative behaviours.

Although, movement into a new area can be motivated by multiple factors, it is unclear why the use of the upper basin is exclusive to males. The upper basin of Macquarie Harbour is not used in aquaculture and much of it is shallower (< 20 m) than the central and lower areas, creating slightly different and seasonally variable environmental conditions (Ross and MacLeod, 2017). Use of this area could be motivated by access to resources (i.e. prey), or preferential environmental conditions (i.e. improved DO and/or preferred thermal conditions). However, this seems unlikely given that in such case, females would be expected to take advantage of the area as well. It has previously been hypothesised that female *S. acanthias* segregate from males to avoid reproductive harassment (Dell'Apa et al., 2014). Our results contradict this as it was males rather than females that altered their behaviour by expanding their range and changing their social dynamics. Given that females are larger than males, sexual segregation in this instance may be better explained by size dependent processes like competition (e.g. access to spillover pellets around the aquaculture pens) rather than mitigation of reproductive harassment.

Data from the state-transition models showed that there was a long-term tendency to move towards the central basin even as the extended range of individuals changed, indicating a mechanism of site fidelity. However, directionality of movement was less apparent in short-

term fine-scale movement, likely because individuals had high horizontal mobility and the potential to swim the length of the harbour within several hours. These observations suggest that long-term movement is directional towards high affinity sites, but short-term individual movement is better described as localized roaming behaviour.

The central basin of Macquarie Harbour represents a critically important area for the species, and it is crucial to understand, not only the way in which the species utilizes this space, but also the mechanisms driving site selectivity. This area includes the deeper sections of the harbour (>30 m) and is where the majority of aquaculture occurs. Due to stratification in the water column, marine water entering the harbour during flood tides (salinity >32 ppt) forms a distinct layer at the bottom of the harbour. This bottom layer has thermally stable conditions (~12 ° C) and generally higher DO levels to the poorly mixed waters above (20-40 mg/l) (Ross and MacLeod, 2017). Thus, the deeper areas (>30 m) not only provide access to excess feed from aquaculture (Chapter 3) they may act as an environmental refuge (e.g. temperature, salinity, DO), explaining why the tagged individuals spent >50% of their time occupying an area that represents ~6% of the total surface area of the harbour. Thus, it is possible that these niche constraints may override sex-specific habitat selection or even density dependent effects in shaping the spatial ecology of the species in this unique habitat.

In this study, tagged individuals were shown to remain within Macquarie Harbour for prolonged periods, suggesting that movement into oceanic waters may be restricted by geophysical characteristics of the system. These observations were consistent between all individuals and correspond with catch records from independent sampling (Chapter 3), indicating that the unique space utilization and behaviour patterns reported herein are widespread in the population. No evidence for offsite movement was found, suggesting that for this population, the possibility of using migration as a strategy to deal with sub-optimal conditions appears to be limited. Moreover, their restricted spatial range, compared with migratory populations,

results in an increased vulnerability to localized environmental pressures, such as the ongoing declines in DO and habitat deterioration seen in Macquarie Harbour. Conventional spatial management strategies that focus on the protection of critical areas for important subcomponents of the population (i.e. juveniles or pregnant females) are unlikely to be successful for this population, given the absence of distinct partitioning and the persistent movement of individuals through all areas of the estuary. The spatial data reported in this study should be used to limit fishing effort and methods to which the species is particularly vulnerable (i.e. gillnets) in areas the species is known to prefer, particularly if conditions in the harbour continue to deteriorate. Moreover, due to the unusual water chemistry of Macquarie Harbour and the low productivity of the species, further study of this population may provide insight into the way this species has adapted to environmental change by modifying their behaviour and ecology.

3

Chapter Three: Reproductive Biology and Diet

3.1. INTRODUCTION

The spiny dogfish, *Squalus acanthias,* is a small demersal elasmobranch with an anti-tropical, circumglobal distribution (Fordham et al., 2006). The basic biology of the species has been extensively explored with multiple studies focussing on their reproductive biology (Avsar, 2001, Hanchet, 1988, Kirnosova, 1989, Yigin and Ismen, 2013), diet (Alonso et al., 2002, Avsar, 2001, Domi et al., 2005) movement (Thorburn et al., 2015) and life history (Kirnosova, 1989, Yigin and Ismen, 2013). Like many elasmobranchs, they are particularly susceptible to overexploitation due to their slow growth, high longevity and low reproductive output (Cortés, 2000). However, they are commonly abundant and have been of historical importance to commercial fisheries both directly (i.e. when caught as a target, by-product and/or bycatch) and indirectly (i.e. decreases in prey abundance through fishing) (Taylor et al., 2009), resulting in a decline of several populations and prompting the IUCN to classify the species status as vulnerable throughout their range and as critically endangered in the NW Atlantic (Fordham et al., 2006). These characteristics highlight a need to improve our understanding of their population dynamics to facilitate appropriate management.

Most of the information known about *S. acanthias* comes from the northern hemisphere, with little known about the life-history characteristics of the species in the southern hemisphere. Previous studies have revealed a variety of common traits in the biology and ecology of *S. acanthias* populations. For instance, they are generalist feeders that target prey

opportunistically (Avsar, 2001) and typically have a two year, synchronous and continuous reproductive cycle (Kirnosova, 1989, Fordham et al., 2006). However, intraspecific variation of individual life-history parameters is common amongst elasmobranches (Beamish et al., 2009), including *S. acanthias,* for which diet, fecundity, age at maturity, longevity and reproductive seasonality vary across their range. These differences occur because life-history characteristics are influenced by demographic and environmental processes such as resource availability and density dependent processes (Frisk et al., 2001). Therefore, information derived from one population is not necessarily relevant to others, and locally derived information should be used whenever possible.

Squalus acanthias inhabits the coastal and shelf waters of southern Australia and is common around western Tasmania, including several estuarine systems including Bathurst Harbour (Edgar, 1991) and Macquarie Harbour (Lyle et al, 2014). Macquarie Harbour is a relatively deep (~55 m) ria estuary with a total surface area of ~280 km^2 that is connected to the sea by a narrow and shallow entrance channel (Teasdale et al., 2003). The estuary receives a large influx of freshwater from the surrounding catchment and is heavily stratified, resulting in three distinct layers with a unique physiochemical profile; the surface layer (approximately <5 m) has low salinity with seasonal temperature fluctuations, the middle layer (approximately 5–25 m) is brackish with relatively consistent temperature (~12^{o}C) and an atypical low range of dissolved oxygen (DO) concentrations (<24%), and the deep layer (approximately >25 m) has physio-chemical parameters similar to marine waters with increased salinity and DO levels (typically ~32%) (Carpenter et al., 1991, Cresswell et al., 1989). The harbour receives pollutants from mining runoff and is the site of a large salmonid aquaculture industry. Overall, Macquarie Harbour represents a very different habitat to that typically inhabited by *S. acanthias*.

Although *S. acanthias* has been reported within small estuarine systems elsewhere, the use of such inshore areas is typically seasonal and sex or life-history stage specific (Henderson et al.,

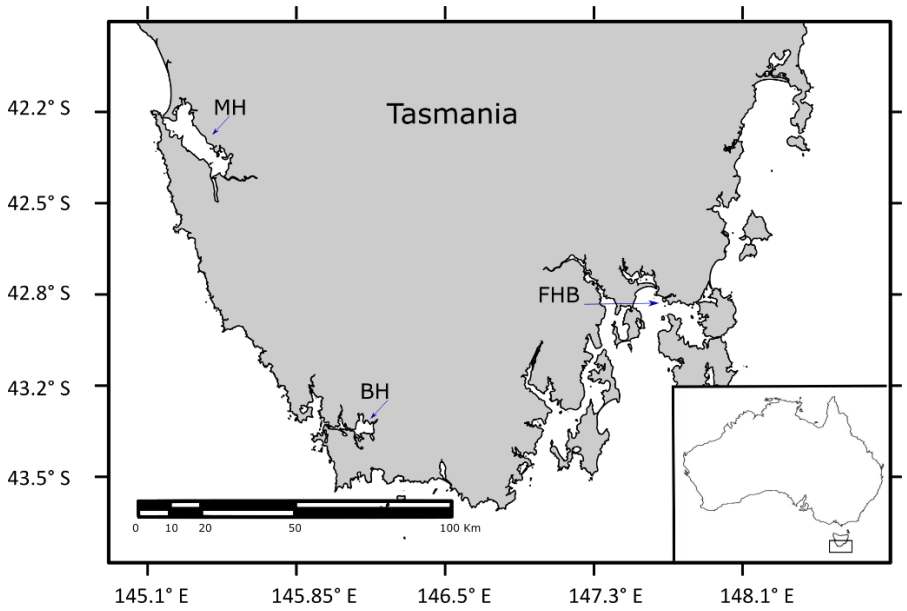

Figure 3.1. Sampling locations for *Squalus acanthias* in Tasmania. Macquarie Harbour (MH), Bathurst Harbour (BH) and Frederick Henry Bay (FHB).

2002). Recent tracking studies in the northern hemisphere indicate that some populations thought to be migratory have been reclassified as resident (Carlson et al., 2014). However, residency in these situations has been defined as permanence within a vast area (1000s km^2 e.g. Bay of Fundy), and seasonal movement patterns still occur within this region (Thorburn et al., 2015). In contrast, adult *S. acanthias* of both sexes have been captured throughout the year in Macquarie Harbour (Lyle et al., 2014). Accordingly, if individuals remain within the confines of Macquarie Harbour for long periods of time, a possibility supported by acoustic tagging (Chapter 2), it would represent site fidelity at a spatial scale not previously reported for the species. Such geographic isolation is rare in species with high mobility potential, but a few cases have been documented. For example, individuals of an isolated sawfish (*Pristis pristis* reported as *P. perotteti*) population remains resident in Lake Nicaragua throughout their entire life cycle (Thorson, 1982), with this population exhibiting reduced fecundity and later maturation than other populations of the same species (Moreno, 2012). These traits make the population particularly vulnerable and accounting for these irregularities proved essential for effective management (Thorson, 1982).

The primary goal of this chapter was to investigate aspects of the reproductive biology and feeding ecology of an elasmobranch species with a conservative life history (*S. acanthias*) and how these may be influenced by an atypical habitat (Macquarie Harbour) and interactions with aquaculture. Given that the species are opportunistic feeders, it was hypothesised that the diet of the population is likely to include by-products from aquaculture such as spillover pellets or escapees. It was also hypothesised that the atypical environmental conditions in the estuary and potential provisioning of food by aquaculture might have an impact on fecundity, size at maturity and timing of the cyclic reproductive patterns compared with other populations of the species living in non-impacted areas. Weight-length, length-length and reproductive parameters for the population are reported, along with data on their diet.

3.2. MATERIALS AND METHODS

3.2.1. Sampling and data collection

Squalus acanthias were sampled during fishery independent surveys conducted at approximately three-month intervals (i.e. seasonally) in Macquarie Harbour (Figure 3.1) between October 2013 and February 2015, totalling six seasonal sampling events. They were captured using a fleet of 50 m monofilament gillnets (114 mm stretched mesh size) and bottom-set demersal longlines (50 baited 1/0 hooks per 250 m longline, squid used for bait). The gear was deployed during daylight hours at depths ranging from 2-42 m and set durations were limited to between 1–3 h to maximise post-capture survival. Individuals were sexed, and length was measured to the nearest mm (fork length, see below). Clasper calcification was assessed in males as an indicator of sexual maturity, and clasper length (CL) was measured from the joint of the anal fin and pelvic girdle to the tip of the clasper. Individuals were considered mature if claspers were fully calcified, maturing if partially calcified and immature if uncalcified (Bubley et al., 2013). A sub-sample (~15 of each sex) was retained during each seasonal survey for biological examination. Size, sex and clasper condition data were supplemented with

information from an earlier study conducted between 2011 and 2013 in Macquarie Harbour using the same gillnet gear (Lyle et al., 2014).

A small number of individuals were also sampled opportunistically from two Tasmanian locations, namely Frederick Henry Bay, a large coastal embayment in south-eastern Tasmania, and Bathurst Harbour, a south-western Tasmanian estuary (Figure 3.1). Sampling in Frederick Henry Bay was undertaken in March 2015 using the abovementioned longlines and in Bathurst Harbour in February/March 2016 using the abovementioned gillnets and longlines. All Frederick Henry Bay individuals were retained for biological investigation (n=41) while all Bathurst Harbour individuals were measured, sexed (clasper condition noted for males) and released (n=84).

Individuals retained for biological examination were frozen for transport and storage until processing. Specimens were defrosted and weighed whole (TW) to the nearest 0.1 g. Various length measurements have been used throughout the literature and to facilitate comparison with other studies, three different length measurements were recorded to the nearest mm: 1) total length (TL), measured from the tip of the snout to the upper lobe of the caudal fin extended parallel to the body axis; 2) fork length (FL), measured from the tip of the snout to the caudal fork, and 3) precaudal length (PCL), measured from last gill slit to the caudal peduncle.

The classification scheme in Walker (2005) was used to assess internal reproductive characteristics of both sexes (i.e. uterine, ovary and oviducal gland condition of females and seminal vesicle, testis condition and sperm presence). Elasmobranch energy storage occurs almost exclusively in the liver and the relative size of the gonads is related to energy investment in reproduction (Rossouw, 1987). As such, liver and gonad masses were used to calculate gonadosomatic and hepatosomatic indices (GSI and HSI), respectively (measured to the nearest 0.1 g). These indices represent the masses of the gonad and liver as proportions of the individual TW.

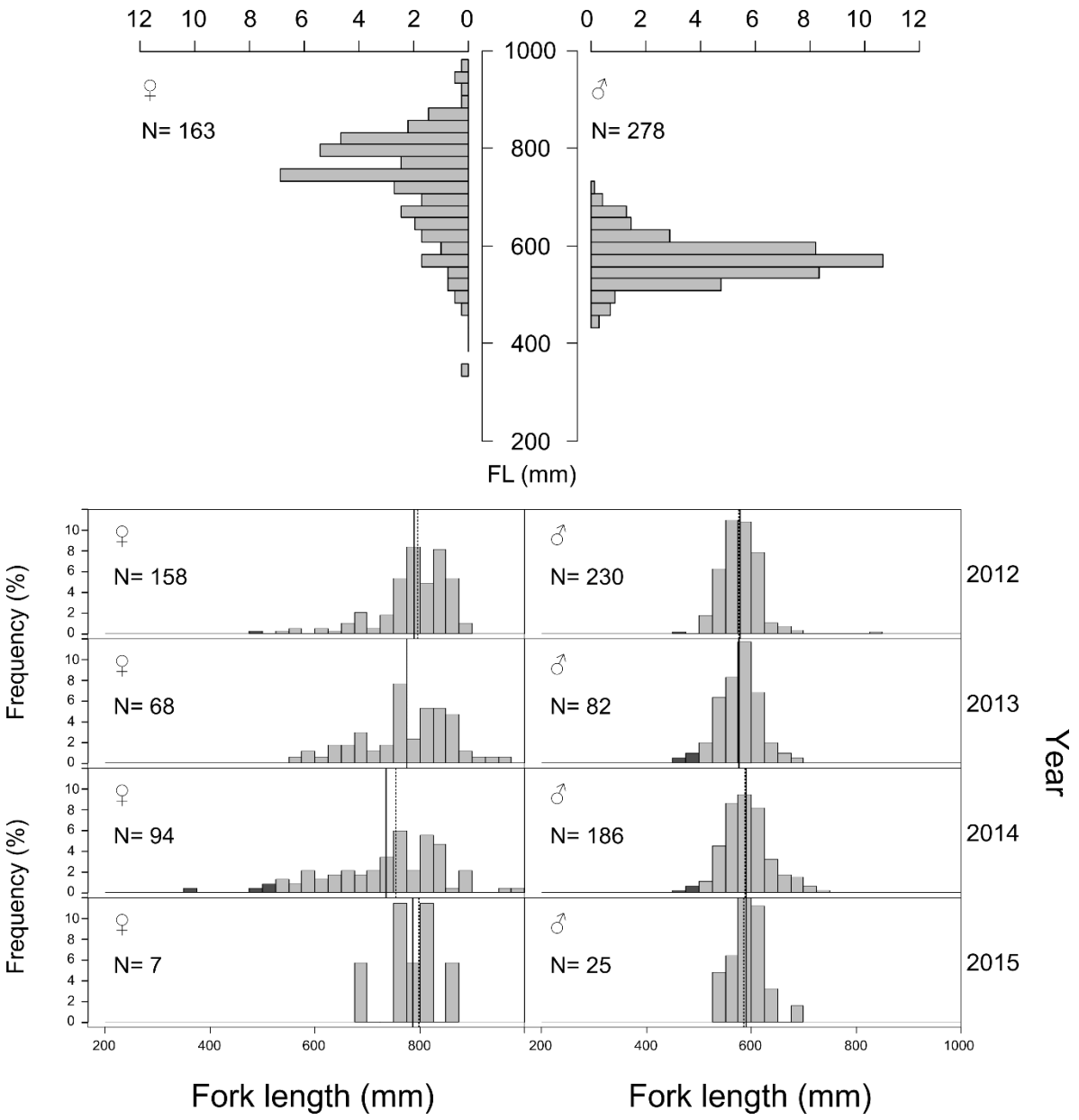

Figure 3.2. Size frequency distribution (%) of male and female *S. acanthias* caught in MH during this study (top) and size frequency by year using data from this study and Lyle et.al. (2014) for MH. N represents the total number of individuals in sample. Individuals below FL_{50} (length at 50% maturity) for each sex are shown for each year (■). The mean (–) and median (···) values for the yearly distributions are shown.

For females, both gonads were removed, and each was opened using a scalpel to remove the individual follicles. All ovarian follicles with diameter >5mm (measured to the nearest mm using electronic callipers) were counted and the maximum follicle diameter (MFD) was recorded. Embryos (TL, TW) or candled eggs (polar diameter) *in-utero* were measured and sexed when possible. Females were considered mature if large yolked ovarian follicles were present (>20mm diameter), if they were pregnant, or if they had a fully developed ovary and uterus (Bubley et

al., 2013). The staging scheme proposed by Tribuzio et al. (2005) for *S. suckleyi* (identical reproductive cycle to *S. acanthias*) was used to classify the reproductive stage of mature females: A) candled eggs present *in-utero*; B-H) visible embryos of varying sizes, or I) post-partum. As this scale made no consideration for resting periods, an additional stage J) was identified for individuals exhibiting the characteristics of a developed reproductive tract but were not pregnant and with no evidence of recent or imminent pregnancy (i.e. no enlarged follicles and no post-partum uterus distention) (as per Bubley et al., 2013).

Diet was explored using stomach content analysis (SCA) and stable isotope analysis (SIA) of $\delta 15N$ and $\delta 13C$ bulk isotope ratios in white muscle tissue. The stomachs of all individuals retained for biological examination were dissected and contents removed and frozen for later examination. White muscle tissue samples were collected from a sub-sample of individuals for SIA and frozen until analysis (see below).

3.2.2. Data analysis

Statistical analysis was performed using the analysis environment R (R Core Team, 2013) and a value of $\alpha=0.05$ was selected *a priori* to indicate statistical significance. Given the limited data available from Frederik Henry Bay and Bathurst Harbour, all subsequent analyses relate to Macquarie Harbour samples unless otherwise specified. The effect of sampling season, gear type and location on mean size was explored by sex using ANOVA. Data conformed to test assumptions and did not require transformation. Individual differences between categories were explored using a *post-hoc* Tukey test. Morphometric relationships between the different length measurements were modelled using linear regression. The coefficients of the resulting predictive models were calculated to facilitate conversion between the different morphometric measurements for sex-specific and pooled data (Appendix 2-Table 1). The length-weight relationship (fitted using non-linear least squares) was established using the power function:

$$TW=\alpha TL^{\beta}$$

where α and β are the regression coefficients. A likelihood ratio test was used to investigate differences in somatic growth between the sexes.

3.2.3. Reproductive biology

Seasonal patterns in HSI, GSI and MFD of mature individuals were explored by using ANOVA. Data conformed to test assumptions and did not require transformation. For both sexes, bilateral symmetry in gonad mass was investigated using a t-test. Size at maturity was assessed by calculating the proportion of mature individuals per size-class (15 mm bins) and fitting a logistic curve to the data to create a maturity ogive. The ogive was used to estimate the size by which 50% (TL_{50}) and 90% (TL_{90}) of the population had become mature. Ovarian fecundity was estimated based on the total number of follicles (>5mm diameter) in both ovaries and uterine fecundity was estimated from the count of either *in-utero* embryos or candled eggs. To determine if reproductive output increased with maternal size, linear regression was used to describe the relationship between ovarian fecundity and length. Data for uterine fecundity at different sizes were insufficient for regressive analysis.

3.2.4. Diet

Stomach contents were examined macroscopically, and prey items were identified to the lowest taxonomical level possible. Aquaculture feed pellets were generally present as a homogeneous mass and therefore individual pellets were not able to be counted. After all identifiable items were processed, the remainder of the stomach content was sieved to extract smaller items. Prey items were counted, weighed to the nearest 0.1 g and submerged in a measuring cylinder to calculate volume to the nearest cm^3. The frequency of occurrence, relative mass contribution and relative volume contribution were calculated for all prey item groups.

Lipids were extracted from white muscle tissue and the remaining muscle freeze-dried, following the manual methodology outlined in Kim & Koch (2011). Freeze-dried samples were ground to a fine powder and 0.4 to 0.7 mg sub-samples weighed into tin capsules using a

microbalance. Carbon and nitrogen stable isotopes were analysed using flash combustion isotope ratio mass spectrometry (varioPYRO cube coupled to Isoprime100 mass spectrometer) (Hussey et al., 2012). Corrected isotope ratios for common macrofauna in Macquarie Harbour and aquaculture feed pellets as well as baseline levels for the system were obtained from Weltz (2017) (IMAS, PhD thesis).

Source specific contributions to diet based on bulk isotopes ($\delta^{15}N$ and $\delta^{13}C$) were analysed using a Bayesian isotopic mixing model (Parnell, 2016). The sources used in the model were selected *a-posteriori* using the observations from stomach content analysis. Source values were corrected for trophic enrichment using a proxy diet-tissue discrimination factor (DTDF) (Kim et al., 2012). These values were derived from a captive experiment on *Triakis semifasciata*, a temperate coastal elasmobranch. Additionally, a sensitivity analysis was conducted using alternative values of DTDF suggested for use in elasmobranchs (Hussey et al., 2010, Logan and Lutcavage, 2010).

An increased number of sources imposes a penalty on the power of the model, so sources with similar isotopic signatures were grouped. The crustacean group comprised crabs (*Paragrapsus gaimardii*) and the carid shrimp (*Palaemon* spp), and the fish group comprised aquaculture escapee Atlantic salmon (*Salmo salar*) and flounder (*Rhombosolea* spp). The model was implemented using the simmr package (Parnell, 2016) in R (R Core Team, 2013) and isotope levels were plotted to determine if *S. acanthias* fitted within the geometry of the proposed model (Kim et al., 2012).

3.3. RESULTS

3.3.1. Morphometrics

A total of 441 *S. acanthias* were captured in Macquarie Harbour, of which 64% were male and 36% female (Appendix 2 -Table 1). Individuals ranged from 242 mm to 985 mm TL, although the sample was dominated by larger fish and only few smaller fish were present (Figure 3.2).

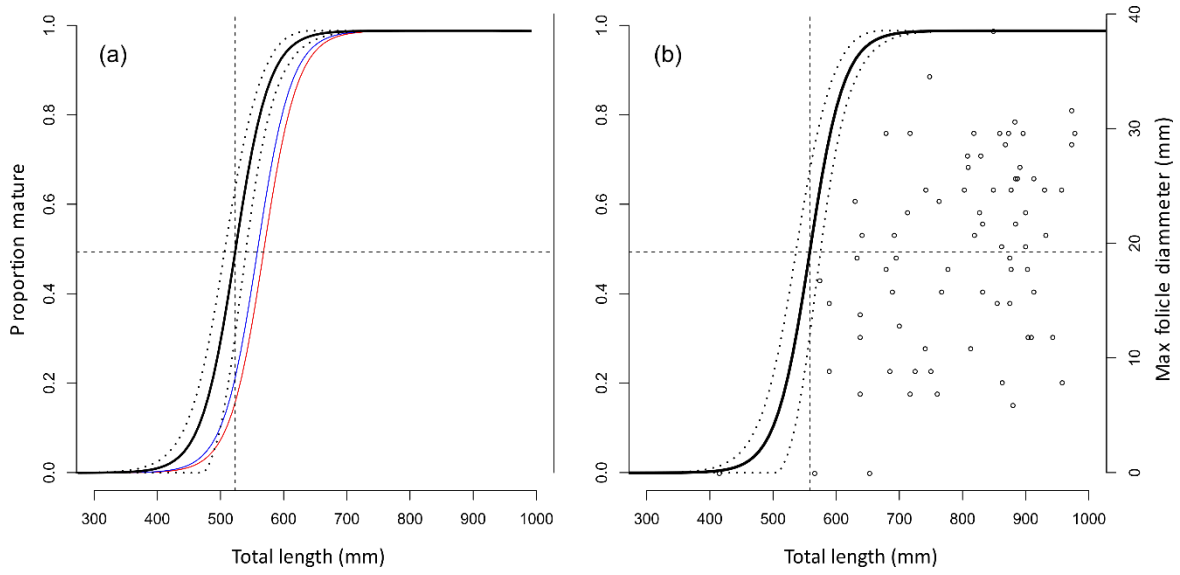

Figure 3.3. (a) Maturity ogives of male *S. acanthias* calculated from Macquarie Harbour (–) +/- S.E. and maturity calculated at other Tasmanian locations BH (–) and FHB (–) . **(b)** Maturity ogives of female *S. acanthias* calculated from Macquarie Harbour +/- S.E . and maximum follicle diameter (MFD) (mm).

Combined size distributions were bimodal and females were on average, significantly larger than males (F = 0.857, p =<0.001). Gear type showed no significant effect on mean size (F = 3.112, p = 0.078), but season did (females F = 2.581, p = 0.02; males F = 4.927, p < 0.001). However, Tukey's pairwise comparisons revealed that in females the result was driven by a single significant but small difference in mean sizes between spring 2013 (mean TL = 880 mm) and autumn 2014 (mean TL = 816 mm); and for males, by smaller mean sizes in summer 2014 compared to each of the other sampling events.

The length measurements TL, FL and PCL were significantly correlated, with adjusted coefficients of determination >90% (Appendix 2-Table 2). The relationship between TW and TL conformed to a power model (Appendix 2-Table 2).

Additional data were available for 408 individuals (F=163, M=245) captured in a previous study conducted in Macquarie Harbour between 2011 and 2013 using the same fleet of gillnets (Lyle et al., 2014). The size structure of samples did not differ significantly between the present study and those in Lyle et al. (2014) (D = 0.077, p = 0.417).

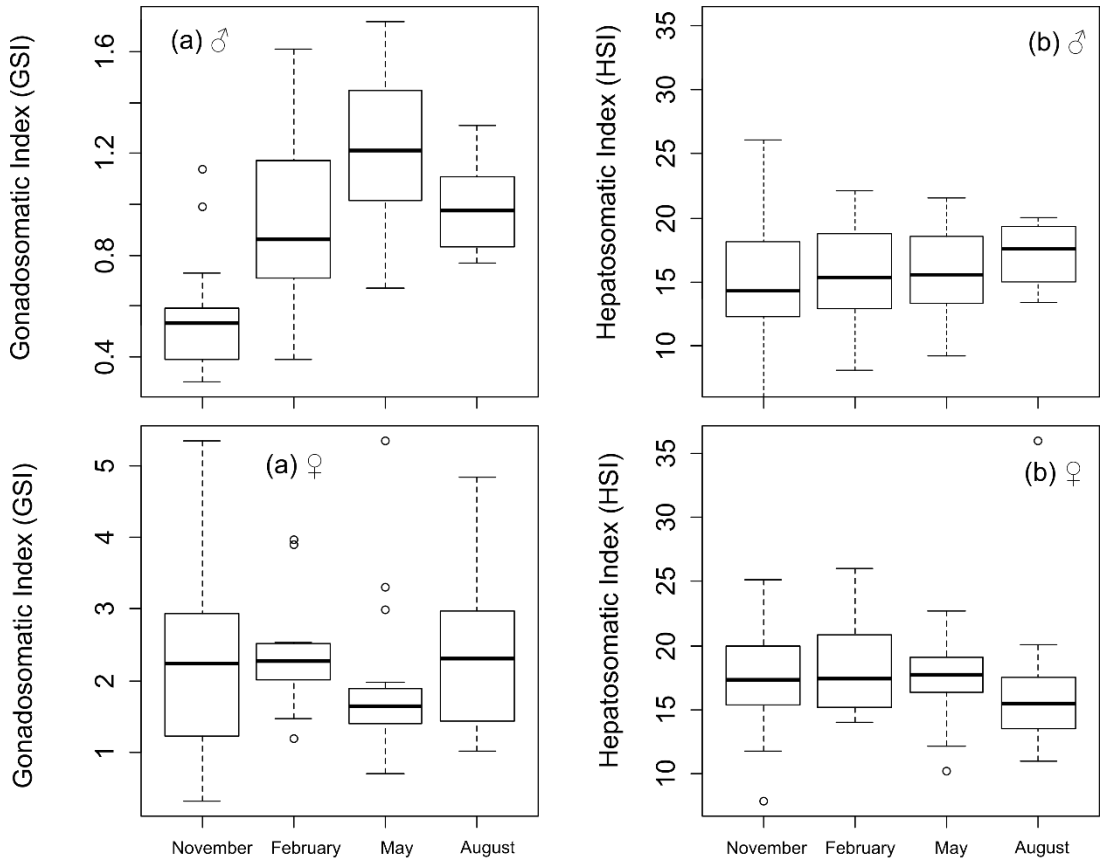

Figure 3.4. Boxplots of gonadosomatic **(a)** and Hepatosomatic **(b)** indices by month for male and female *S. acanthias* captured in Macquarie Harbour by sampling season (November and February pooled data from two years). Boxplot shows median, interquartile range, upper and lower fences and outliers(o)

3.3.2. Maturation

Size at maturity was significantly sexually dimorphic (z= 2.38, p=0.018) with the size at which 50% of the population attained sexual maturity (TL_{50}) being 519 mm TL for males (logistic regression, max likelihood=-6.067, s.e.=0.04, p=0.002) and 559 mm TL for females (logistic regression, max likelihood=-7.810, s.e.=0.01, p=0.001) (Figure 3.3). Male maturity (TL_{50}) occurred at a significantly smaller size in Macquarie Harbour compared with the other Tasmanian sites (estimated based on clasper calcification) (Figure 3.3). Maturity of females could not be determined externally and therefore it was not possible to compare the Macquarie Harbour with other sites.

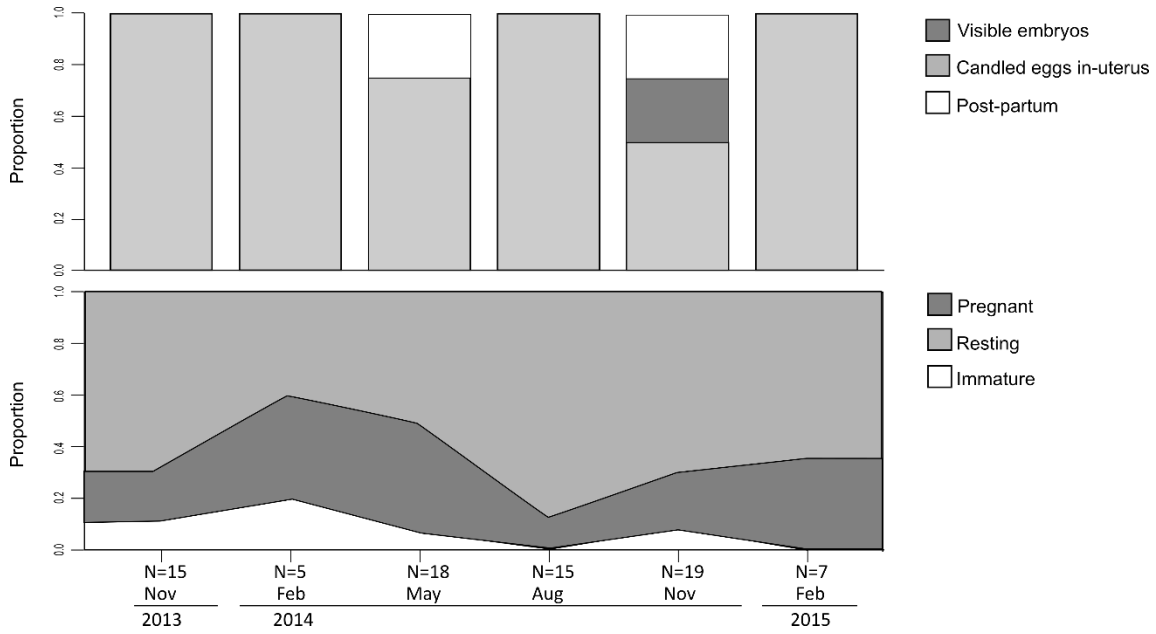

Figure 3.5. **Top**: Seasonal classification of observed pregnancy stages based on the classes proposed by Tribuzio et al. (2009). Observed stages were limited to: candled eggs *in-utero* with no visible embryo, post-partum individuals with enlarged flaccid uteri and visible embryos *in-utero* with no external yolk**. Bottom**: Reproductive status of all sampled females, immature, pregnant or mature with no apparent signs of recent or current pregnancy (resting).

Table 3.1. Ovarian and uterine fecundity of *S. acanthias* in Macquarie Harbour. Ovarian fecundity estimated using ovarian follicles >20mm in diameter. Uterine fecundity estimated from candled eggs and embryos

Size (mm) TL	Number of ovarian eggs (N individuals)																	N	Mean fecundity
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
< 650			1	3		3	1		1									9	6.4
651 - 700			1			1	4											6	7.2
701 - 750					2	1	1			1	1			1	1			8	10.1
751 - 800							2	1		1	1		1					6	10.3
801 - 850		1					1	4	1		2	1						10	9.4
851 - 900			1	1	1	3	2	1	4	2	1	1			1		1	19	9.6
901 - 950		1			2		1	1	2	2								9	8.2
> 950	1		1			1	1					1		1				6	8.2
All	1	2	4	4	5	9	13	7	8	6	5	3	1	2	2	0	1	72	8.7

Size (mm) TL	Uterine eggs or embryos (N individuals)											N	Mean fecundity
	1	2	3	4	5	6	7	8	13				
< - 700			2									2	3.0
701 - 750	2		1									3	1.7
751 - 800	1							1				2	4.5
851 - 900	1	1				1		1	1			5	6.0
901 - 950		3		1								4	2.5
> 950			1									1	3.0
All	4	4	4	1	0	1	0	2	1			17	3.4

3.3.1. Female reproduction

Through the study, a total of 79 female *S. acanthias* caught in Macquarie Harbour were dissected. Five individuals (6.3%) exhibited no signs of gonad development and were judged to be immature. Of the 74 individuals judged to be mature, 22 (30%) were in an active stage of pregnancy. The remainder (52 individuals), while obviously mature (enlarged uteri, oviducal glands and developed ovaries) appeared to be either pre-ovulatory or in a resting state as evidenced by the lack of post-partum conditions, fertilized eggs *in-utero*, or enlarged ovarian follicles. These conditions were observed throughout the year, with 16 individuals (30%) showing no macroscopic signs of reproductive activity and 36 (70%) exhibiting enlarged *corpora atretica* (>20 mm) in varying states of reabsorption.

A lack of seasonal variation in GSI, HSI or MFD as well as the presence of pre-ovulatory and newly pregnant females during all seasons meant that the timing of parturition, mating and seasonality of the reproductive cycle could not be determined. This information suggests that females can be reproductively active/inactive at any time of the year. Although pregnant females were present throughout the year, with the exception of a single individual, all were in the same developmental stage (candled eggs with no visible embryo). Only one individual was in an advanced stage of pregnancy (caught November 2014), with three near-term embryos with no visible external yolk sac. Furthermore, an individual caught in May 2014 and two others caught in November 2014 had enlarged uteri suggesting they may have been post-partum or had possibly aborted advanced stage embryos due to capture stress (Figure 3.5). As early stages of pregnancy were observed in all sampling periods, mating likely occurred throughout the year (assuming no storage of sperm) (Figure 3.5).

Ovarian fecundity based on follicles > 20 mm diameter ranged from 3 to 18, with a mode of 8, and was significantly correlated with maternal length ($n = 71$, $F=9.81$, $p=0.01$, R^2 adj$=0.11$) (Table 3.2). There was no evidence of bilateral asymmetry in gonad mass ($t = -1.235$, $p = 0.22$) or ovarian

Table 3.2. Dietary composition of *S. acanthias* based on stomach contents as measured by proportional mass and volume contribution and frequency of occurrence for 44 individuals that contained food items. Values were estimated based on all prey items (total) and excluding aquaculture pellets (natural). Frequency of occurrence (FO) refers to the proportion of different individuals in which a specific prey item can be found out of the total individuals with non-empty stomachs. Natural items are the recalculated values when excluding aquaculture pellets

		Total			Natural	
		Weight (%)	Volume (%)	FO (%)	Weight (%)	Volume (%)
Elasmobranchii	*S.acanthias*	<0.9	<0.9	4.55	<0.9	<0.9
Teleost	*Salmo salar*	3.36	4.3	20.45	53.96	53.88
	Unidentified sp.1 (small)	<0.9	<0.9	2.27	0.97	1.83
	Unidentified sp.2 (large)	2.73	3.28	6.82	43.78	41.1
Crustacea	*Paragrapsus gaimardii*	<0.9	<0.9	2.27	<0.9	0.91
	Palaemon spp.	<0.9	<0.9	27.27	<0.9	0.91
Bivalvia	*Xenostrobus securis*	<0.9	<0.9	13.64	<0.9	0.91
Annelida	Unidentified	<0.9	<0.9	2.27	<0.9	<0.9
Aquaculture feed	Pellets	93.77	92.02	77.27		

fecundity (t = 1.996, p = 0.63). Uterine fecundity ranged from 3 to 13 eggs/embryos with a mode of 4. Based on the size of the three near-term embryos (219 mm, se=4.72 mm) size at birth is approximately 220 mm TL. Embryos were deemed to be near term based on the yolk sac being drawn into the body (Frederick et al., 1947).

3.3.2. Male reproduction

There was no bilateral asymmetry of male gonad masses (t = -0.145, p = 0.88) and GSI appeared to exhibit a cyclic pattern that was highest during late autumn (May) and lowest during spring (November) (Figure 3.4). However, semen was present in the ductus deferens, seminal vesicles and sperm sac of all mature individuals throughout the year.

3.3.3. Diet

Of the 150 individuals analysed for stomach contents, 68% had an empty stomach. The prevalence of empty stomachs varied between sampling events, from 80% (November 2014) to 31% (February 2014). Digestion, liquefication and damage made individual counts impossible for most food items, especially small prey. Salmonid feed pellets were the main dietary

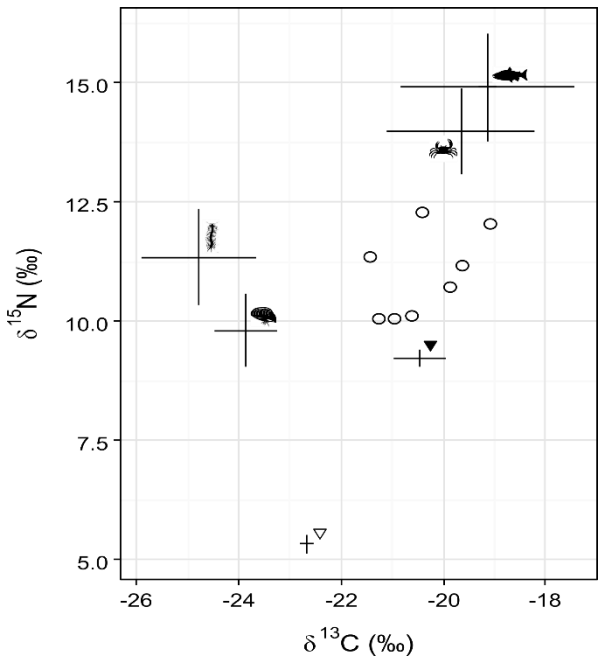

Figure 3.6. Values of $\delta^{15}N$ and $\delta^{13}C$ for *S. acanthias* (o) in Macquarie Harbour and mean source values corrected for enrichment (crustaceans, fish, mussels, ▼ pellet type 1, ▯ pellet type 2 and annelids).

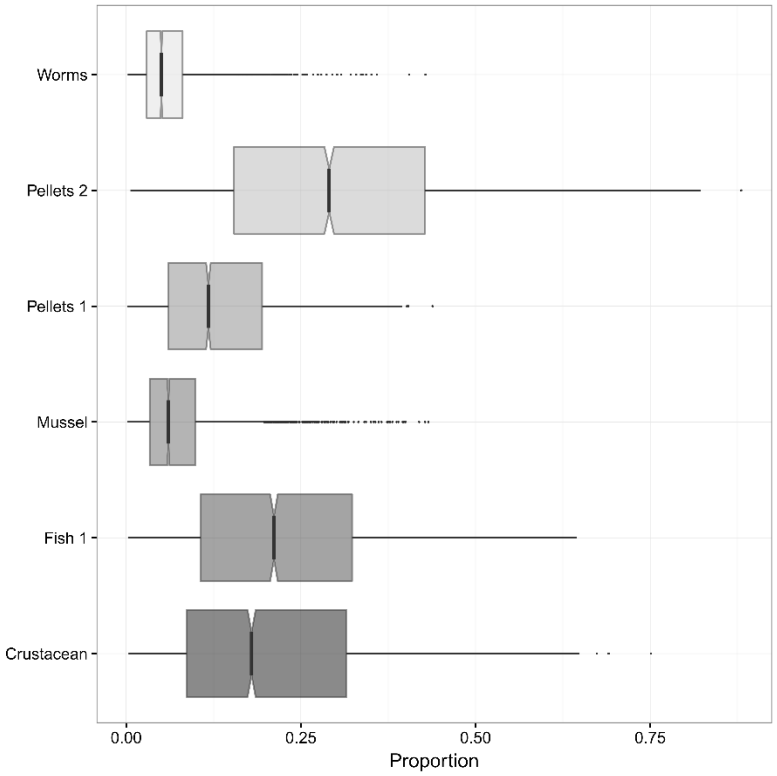

Figure 3.7. Density distribution (quantiles) of the projected dietary contribution of prey items to the diet of *S. acanthias,* based on isotopic Bayesian mixing model

contributor based on weight, volume and frequency of occurrence (Table 3.3). Carid shrimp were the only other item to occur in all sampling seasons and were the second most commonly found item. Despite this, due to their small size, they were not important in terms of weight and volume. Small Mytilids (*Xenostrobus securis*) and Atlantic salmon (*Salmo salar*) were recorded in multiple individuals but only during a single sampling period, November 2013 and February 2015, respectively. Plant material was present in several stomachs but was likely to have been ingested incidentally and as it is unlikely to provide nutritional value it has been excluded from analyses. Contribution indices excluding feed pellets were also calculated (n= 29 individuals), showing that teleosts contributed the most by weight and volume, although this was largely the result of high volumes of Atlantic salmon (6 out of 8 individuals) found during an escape event in February 2015. Salmon in stomachs were found as bite sized chunks rather than whole fish. Prey items identified in the stomachs were used to select the sources to be included in the isotopic mixing model (Figure 3.6). Two types of pellets used by different aquaculture companies were also included as source items. DTDFs in Kim et al. (2012) did not result in a violation of the model assumptions ($\delta^{15}N$ =3.7% ±0.2% and $\delta^{13}C$= 1.8% ±0.5%).

After correction for trophic enrichment, the isotope values of *S. acanthias* were within the geometry of the source items, suggesting that the sources selected could adequately explain the isotopic concentrations in *S. acanthias* (Figure 3.6). The pellets accounted for the greatest proportion of the diet with a mean contribution of 42.7% (Pellet 1 = 13.5%, Pellet 2= 29.2 %) (Figure 3.7). The isotopic signature of two source pairs showed a high correlation, fish and crustaceans (R^2 = 0.73) and the two pellet types (R^2=0.77) (Figure 3.6). This reduced the discriminating power of the model for the individual items in each pair, and it is possible that the dietary contributions of those items are somewhat underestimated by the mixing model.

3.4. DISCUSSION

The reproductive cycle of *S. acanthias* in Macquarie Harbour does not conform to the known reproductive model of the species (Bubley et al., 2013) and may be affected by resource limitation, spatial isolation and potentially environmental stressors. Unlike other populations, reproduction does not appear to follow a clear seasonal pattern, and females appear to have a protracted resting period, with only about a third of individuals pregnant, most at an early stage, suggesting that only a portion of the mature individuals in the population are reproductively active at any given time. Stomach content and stable isotope analyses produced consistent results, showing that diet was dominated by provisioning from aquaculture with no evidence for immigration from outside the harbour. These observations highlight unique life-history and ecological traits of *S. acanthias* in Macquarie Harbour.

Squalus acanthias in Macquarie Harbour mature at smaller sizes than most other populations (Chapter 4), a trait that seems to be specific to this area and not widespread in other Tasmanian populations. Size at maturity (TL_{50}) was 519 mm for males and 559 mm for females, lower than values observed for the other Tasmanian areas, Frederick Henry Bay (males = 560 mm, this study) and Bathurst Harbour (males = 565 mm, this study) or the nearest studied population in New Zealand (males =580 mm, females = 745 mm) (Hanchet, 1988). Despite the differences in size at maturity, individuals in Macquarie Harbour appear to grow to similar sizes than other populations, but at a faster rate (see Chapter 4), suggesting that this population is likely mature at a younger age. It is important to note that although the smaller mature males captured in Macquarie Harbour were well below the size at maturity reported elsewhere (Bubley et al., 2013 and references therein), the paucity of juveniles in the data resulted in relatively high uncertainty for the male maturity ogive in Macquarie Harbour. Therefore, it is possible that they could mature at even smaller sizes than shown by the analysis, so that male size at maturity

reported herein may be an overestimation and should be considered as the maximum value until further data can be collected to improve precision of the maturity ogive.

As in other elasmobranchs, intraspecific variation of reproductive parameters *is* common in *S. acanthias*, presenting as differences in age at maturity, fecundity and seasonal timing of the reproductive stages between populations. However, several reproductive characteristics of *S. acanthias* in Macquarie Harbour appear to deviate from the typical pattern. The species normally has a continuous and synchronous reproductive cycle, for which gestation lasts 23 months (e.g Avsar, 2001, Hanchet, 1988, Jones and Ugland, 2001, Kirnosova, 1989, Yigin and Ismen, 2013). This cycle is linked to season-specific patterns of movement, including sexual segregation and ontogenetic or reproductive cycle specific movements (e.g. female inshore movement after mating) (Hanchet, 1988). Vitellogenesis and embryonic development occur seasonally (Waltrick et al., 2012), whereby ovulation occurs shortly after parturition, with no rest between subsequent pregnancies (Avsar, 2001), resulting in mature females being at one of two reproductive stages at all times (e.g. when one half of the population is ovulating, the other is pregnant and beginning the second year of gestation)(Carrier et al., 2012).

Our results did not conform to either of these conditions. A large proportion of the females examined (70%) were neither pregnant nor displayed post-partum conditions regardless of time of year. This suggests the existence of a protracted resting period between pregnancies, a phenomenon that has only been reported in *S. acanthias* for few individuals in New Zealand (Hanchett, 1988) and once in the sister species *S. suckleyi* in the Northeast Pacific (Tribuzio and Kruse, 2012). Furthermore, all but one of the pregnant females examined was in an early stage of pregnancy, with candled eggs with no visible embryos. This candled egg stage is known to occur immediately after ovulation and lasts approximately four months, after which the candle ruptures when the embryo reaches ~6-7 cm in length (Demirhan and Seyhan, 2007). Thus, the presence of newly pregnant females throughout the year indicates that in Macquarie Harbour

S. acanthias may have an asynchronous reproductive cycle, which has not previously been reported for the species.

Male GSI appeared to be seasonally variable, with highest values in May (autumn) and lowest in November (spring). In Macquarie Harbour, male and female *S. acanthias* do not fully segregate, although intersex association does decrease during winter (Chapter 2). Sperm production was observed throughout the year in all mature individuals examined, suggesting that the seasonal interruption in spermatogenesis does not occur in Macquarie Harbour. This pattern differs from previous reports of the male reproductive cycle (Simpson and Wardle, 1967) where males and females spatially segregate after mating and then male gonads decrease in volume as spermatogenesis is halted (Chatzispyrou and Megalofonou, 2005). Coupled with the presence of newly pregnant females throughout the study, these observations are evidence that some mating activity likely occurs throughout the year. Notably, the lowest values of male GSI occurred at the times of highest association between the sexes (i.e. summer) and presumably a time of peak mating activity, corresponding with a slight dip in female GSI. As such, the peak in male GSI values observed in Macquarie Harbour most likely reflect sperm accumulation due to reduced mating activity during times of lower intersex association.

The effective absence of pregnancies at stages other than candled eggs could be explained by a variety of factors. Late-term, stress-induced abortions can occur during sampling and are affected by factors such as gear type and soak time, which can cause an underrepresentation of gravid females (Adams et al., 2018). However, large-scale capture induced abortions were not obvious in this study with only 4% of mature females examined displaying an expanded uterus consistent with post-partum conditions that may have indicated an abortion or recent birth. Alternatively, the observed pregnancy stage distribution could be explained by life-cycle specific migratory behaviour. For example, female *S. acanthias* in New Zealand move offshore during summer where mating and ovulation occurs (four-month period elapses from mating to the

rupture of candled eggs), after which they move inshore during gestation (Hanchet, 1988). In Macquarie Harbour, all acoustically tagged adults remained resident within the system for 18 months of uninterrupted detection (Chapter2). Within this timeframe, any reproductive cycle driven emigration would have likely been observed regardless of the reproductive status of the females when tagged. While the suggestion that pregnant females leave the harbour cannot definitively be refuted, the observations from this study and acoustic tracking information suggest that emigration is uncommon and *S. acanthias* complete their full life cycle within the harbour. Thus, if pregnant individuals do not migrate out of the system, it is feasible that the lack of advanced stage pregnancies indicates that embryos are being aborted or embryonic development is being suppressed.

Environmentally mediated reproductive suppression is a widespread survival adaptation common across vertebrates, including elasmobranchs (Walker, 2005). Reproductive suppression usually occurs during early-pregnancy (i.e. when the energetic investment is minimized), when the individual is exposed to unfavourable conditions which are unlikely to improve (Ward et al., 2009). A further possibility is obligate diapause, which allows individuals to delay embryo development (Waltrick et al., 2012). Wyffels (2009) suggested that diapause in elasmobranchs can be identified by the occurrence of eggs *in utero* for extended periods. This definition is consistent with our observations, however, diapause has not been previously reported in *S. acanthias* and a resumption of development was not observed.

Regardless of the mechanism, if the patterns observed in Macquarie Harbour are the result of reproductive suppression, the widespread lack of later stages of pregnancy seen in this study are cause for concern, as this would suggest that the population could be severely compromised reproductively. Reproductive characteristics of elasmobranchs can be affected by environmental factors or density dependent processes that result from exploitation or extreme competition (Cortes, 2004). The extent of the irregularities observed in the Macquarie Harbour

population suggest a more direct mechanism, such as environmental constraints or food resource limitation. Interestingly, there was a much higher incidence of empty stomachs in Macquarie Harbour (62 %) when compared to other populations like those in New Zealand (29%) (Hanchet, 1991) and Argentina (9%) (Alonso et al., 2002). The resulting nutritional distress in *S. acanthias* could explain the reproductive irregularities suggested in this study. Widespread reproductive failure linked to nutritional stress has been reported in other marine vertebrates such as penguins, seals and killer whales (Ward et al., 2009). In mammals, starvation causes abortions through ketoacidosis due to ketone-bodies adversely impacting the developing embryos (Sinha et al., 2014). Interestingly elasmobranchs have been recently found to produce ketone bodies in quantities similar to mammals during starvation (Dove et al., 2012, Valls et al., 2016) although such mechanisms remain largely unstudied in most species, including *S. acanthias*.

The broad geographic range of *S. acanthias* means diet varies between populations and is determined by local prey availability. Macquarie Harbour is naturally depauperate (Edgar et al., 1999), so it was expected that the diet of the species in the area would be low in diversity and dominated by local invertebrates. Surprisingly, the diet of individuals examined in the present study was overwhelmingly dominated by spillover pellets throughout the year, with natural prey items playing a secondary role. It is possible that the capture of prey is more energetically demanding than feeding on pellets from the substrate, particularly when prey species are sparse. Feeding of the species on aquaculture pellets has been reported in Chile (Gaitan-Espintia et al., 2017), where, similar to this study, individuals had a low diversity diet. In contrast to Macquarie Harbour, individuals in Chile had a much lower incidence of empty stomachs (31.5%) and natural prey items were more commonly ingested (i.e. hake and anchovy), suggesting that although that population fed opportunistically on pellets, they are not reliant on them as a primary nutritional source.

Interestingly, in Macquarie Harbour one type of pellet was estimated to contribute twice as much as the other, although it is unlikely that *S. acanthias* would favour one type of feed. Operating area, biomass production, feed inputs and feeding regimes differ between each of the three aquaculture companies operating in Macquarie Harbour. This likely impacts the quantities of over-feed reaching the substrate of each company and could explain the greater contribution of one type of pellet over the other. Furthermore, two of the non-pellet prey items that were part of *S. acanthias* diet are also linked to aquaculture operations, namely the Atlantic salmon (escapees) and bivalves detached from cages during cleaning (although they can also occur at sites away from the farms). These observations suggest that the diet of *S. acanthias* in Macquarie Harbour is largely subsidised by nutrients provided by aquaculture.

The dietary reliance on aquaculture in Macquarie Harbour is unlikely to be temporary or recently developed. Aquaculture companies have operated continuously in Macquarie Harbour since the early 1990s (Ford, 2017). As operations have expanded, they may have provided an important alternative food source to *S. acanthias*. It is also feasible that the current abundance of the species within the system is likely a result of this nutritional subsidy. However, changes in regulation and improvements in aquaculture practices have greatly reduced the amount of waste feed produced by industry despite increased biomass of salmonids (Kirkpatrick et al., 2017). The resulting drop in standing pellet biomass could explain the elevated proportion of empty stomachs seen in Macquarie Harbour, and because there does not currently appear to be alternative natural prey to sustain the population, it is likely that the population is now becoming resource-limited, possibly impacting processes such as reproduction success.

The impact of marine cage aquaculture has on wild fish populations remains poorly understood, although recent studies show that these interactions can affect populations in important ways. For example, wild saithe (*Pollachius virens*) associated with aquaculture farms in Norway altered their migratory patterns and showed a higher level of philopatry around the farms than is typical

(Otterå and Skilbrei, 2014). Likewise, feeding on lipid-rich pellets in the short-term has been reported to cause phenotypical differences (e.g. differences in otolith growth) (Abaad et al., 2016, Fernandez-Jover and Sanchez-Jerez, 2015) and altered reproductive characteristics (Uglem et al., 2014) in wild populations. Feed pellets are designed to maximize growth of the cultured species and may contain antibiotics, or hormonal supplements, however, the long-term health effects of such a diet are unknown (Arechavala-Lopez et al., 2015). Therefore, some of the anomalies in the spatial utilisation and reproductive cycle of *S. acanthias* could be caused by a long-term pellet dominated diet.

Dissolved oxygen (DO) concentrations declined sharply throughout much of Macquarie Harbour from about 2009 on, corresponding with expansion of the aquaculture industry (Figure 1.3) (Ross and MacLeod, 2017). Spillover pellets and fish waste are rich sources of organic carbon, which increases bacterial activity and results in a higher oxygen demand (Mente et al., 2006). These issues are exacerbated in Macquarie Harbour where water exchange is restricted, resulting in naturally low oxygen conditions (Cresswell et al., 1989). Macquarie Harbour has a low diversity of predatory fish, with *S. acanthias* and the endangered Maugean skate (*Zearaja maugeana*) being among the most abundant large predatory species (Lyle et al., 2014). Dietary analysis of *Z. maugeana* revealed that the skate does not feed directly on pellets (Bell et al., 2016). Considering the abundance of *S. acanthias* and the importance of pellets in the diet, it is likely that the species perform an important ecological service by removing large portions of over-feed that would otherwise be broken down by aerobic sedimentation processes, thereby further depleting oxygen levels (Fernandez-Jover et al., 2008).

In response to the ongoing environmental degradation of Macquarie Harbour, restrictions have been imposed that reduce the allowable aquaculture biomass (DPIPWE, 2013). Furthermore, the companies are trialling the implementation of waste capture devices that would limit the spillover of uneaten pellets. Low DO conditions in the harbour could be linked to large biomass

declines in benthic communities seen in recent years (Ross and MacLeod, 2017) and if continued could further limit the availability of natural prey to *S. acanthias*.

3.4.1. Conclusion

Results of this study indirectly support the notion that *S. acanthias* in Macquarie Harbour could be a semi-isolated, or isolated population, which means that they may be particularly vulnerable to disturbances due to their restricted range. Likewise, this work revealed that there is a strong interaction between the finfish aquaculture operations and *S. acanthias* due to the provisioning of spillover feed. By exploiting this resource, they are likely providing an important ecological service by removing over feed that would otherwise be broken down by aerobic sedimentation processes. However, the full effect on the species is unknown and it is possible that some of the observed reproductive anomalies could be due to the heavy reliance on aquaculture feed, whether directly due to the feed composition, or indirectly due to the inability of the feed to support the population (i.e. resource limitation). At this time, it is unclear if these characteristics are the result of a short-term response to prevailing conditions, however, if conditions remain unchanged, these relationships are likely to shape the future of this seemingly unique population. Therefore, future work should focus on improving our understanding of the complex mechanistic links between environmental or dietary conditions and the reproductive cycle of *S. acanthias*. Furthermore, while these traits are potentially exclusive to Macquarie Harbour, additional work is required to determine how they compare with other Australian populations. Given the relatively high abundance of the species in Macquarie Harbour, they may be an ideal indicator to monitor the health of the system in response to changes in the environment and the aquaculture operations.

4

Chapter Four: Age and Growth of *S. acanthias* Using Vertebrae and Dorsal Spines

4.1. INTRODUCTION

The spiny dogfish (*Squalus acanthias*) is an abundant elasmobranch in temperate waters of the Atlantic and South Pacific Oceans (Fordham et al., 2006). Like many elasmobranchs they have a conservative life-history strategy (i.e. slow growth, longevity and a low reproductive output) (Fahy, 1988) and are therefore particularly susceptible to fishing pressure and environmental change (Dulvy et al., 2008). Intense fishing of some northern hemisphere populations, has resulted in large declines in biomass (Taylor et al., 2009) with Northeast Atlantic stocks listed as critically endangered and the species classified by the IUCN as vulnerable globally (Fordham et al., 2006). Although relatively common around southern Australia, the species is not the target of fisheries and their life-history characteristics remain largely unstudied.

Squalus acanthias inhabit coastal areas and embayments around Tasmania (Last and Stevens, 2004). One such location is Macquarie Harbour, an estuarine inlet in western Tasmania, where *S. acanthias* is the most abundant elasmobranch species present (Lyle et al. 2014). The harbour is the site of large-scale salmonid aquaculture operations and *S. acanthias* are occasionally taken as bycatch in a recreational gillnet fishery targeting salmonid escapees (Lyle et al., 2014). The impact of the fishery is expected to be relatively small since fishers tend to avoid fishing in areas where the species is most common (Chapter 2) and, if captured, most are released or discarded (Lyle et al., 2014). However, post-release survival is likely to be moderately low (Bell and Lyle,

2016, Braccini et al., 2012) and therefore there is some incidental mortality associated with the fishery.

Recent observations on *S. acanthias* in Macquarie Harbour suggest that aspects of their diet, reproduction (Chapter 3) and spatial ecology (Chapter 2) have been impacted by the unique physio-chemical characteristics of the harbour and/or aquaculture activities. Of main concern is aquaculture provisioning of spill over pellets and decreased dissolved oxygen (DO) concentrations in the mid to deepest depths of the harbor. Considering the low productivity of the species (Cortés, 2000) and the high affinity to the harbour seen in adults (see Chapter 2), it is likely that *S. acanthias* in Macquarie Harbour are particularly vulnerable to exogenous factors, both natural (i.e. environmental) and anthropogenic (i.e. aquaculture and fishing). Since historical information on any of the Tasmanian stocks of *S. acanthias* is lacking, current age and growth information will be crucial to inform future management and may provide insight into the conditions that resulted in the development of these traits.

Age structure and growth (estimated from age-length data) are two of the most valuable characteristics in the biology of elasmobranchs, as they can be used to estimate natural mortality, longevity, productivity, resilience and age of maturation (Frisk et al., 2001). These in turn can inform stock assessment and demographic models, which are useful for management and conservation of a species. As is the case with many elasmobranchs, growth characteristics of *S. acanthias* vary spatially in response to environmental, biological and density dependent processes (Bubley et al., 2012). For example, fishing pressure has altered the population dynamics of *S. acanthias* in the North Atlantic, resulting in slower growth and earlier maturation than in pre-fishing conditions (Taylor and Gallucci, 2009). Thus, it would be inappropriate to use growth characteristics of a different population to infer the demographics of *S. acanthias* in Macquarie Harbour.

In addition to variability in growth characteristics between populations, some of the reported variability is likely caused by methodological inconsistencies in age determination (Beamish et al., 2009). Traditionally, *S. acanthias* have been aged using the second dorsal spine (Taylor et al., 2013). The spine is a calcifying structure with annual accretionary growth, which has been validated using chemical tagging and bomb radiocarbon (Jones and Geen, 1977, Tucker, 1985, Campana et al., 2006). However, the spine is exposed to environmental wear which can result in ages being underestimated, particularly in older individuals (Beamish et al., 2009). A comparative study between ageing laboratories linked this issue to the introduction of systematic bias in ageing studies of *S. acanthias* (Taylor et al., 2013). Although internal structures such as vertebrae are commonly used in ageing studies of elasmobranchs because they are not subject to breakage and wear, their use for age determination in *S. acanthias* has been traditionally avoided due to poorly defined growth increments. However, Bubley et al. (2012) demonstrated that *S. acanthias* vertebrae treated with a modified histological staining method can be accurately aged, providing an alternative approach for age determination in the species.

Somatic growth is commonly described by fitting a simple mathematic function to age-length data, typically the von-Bertalanffy growth function (VBGM) (Cailliet et al., 2006). However, there are numerous cases where data support the use of a different growth trajectory (Pardo et al., 2013). An emerging practice has been the evaluation of multiple candidate models (e.g. VBGM, Gompertz, Logistic growth models) and alternate parameters (e.g. 2 or 3 parameter versions of VBGM) or model structures (e.g. biphasic growth models) (Araya and Cubillos, 2006). Using the Akaike information criterion (AIC) to evaluate model parsimony (i.e. balance between model complexity and goodness of fit), a 'best' model can be selected from the candidates (Cailliet et al., 2006). More recently, the notion of a 'true' growth model has been replaced for a probabilistic approach using multi-model inference (MMI) based on information theory (Katsanevakis and Maravelias, 2008). A MMI approach treats Akaike weights as the

relative probability of the different candidate models that together can be considered to be an *a-posteriori* probability distribution to calculate model-averaged growth parameters (Katsanevakis and Maravelias, 2008). This approach reduces the uncertainty associated with model fit and has been shown to provide robust parameter estimates in data poor situations (Thorson and Simpfendorfer, 2009, Smart et al., 2013).

Past and ongoing anthropogenic stressors (i.e. aquaculture) have been linked with deteriorating environmental conditions in Macquarie Harbour, raising important management concerns for the native fauna, including *S. acanthias*, although to date, focus on the species has been limited. Further, management is impeded by the gap in knowledge about the local life-history characteristics of the species, in particular age and growth, which are key inputs into understanding demographic structure and population status. The overall aim of this component of the study was to determine the age and growth characteristics of *S. acanthias* in Macquarie Harbour by establishing a reliable ageing protocol through the comparison of vertebrae and dorsal spines as well as using a MMI framework to improve growth model selection.

4.2. MATERIALS AND METHODS

4.2.1. Sample collection

Squalus acanthias were obtained from Macquarie Harbour during fishery independent surveys conducted at approximately three-monthly intervals from November 2013 to February 2015 (total of six sampling events) (Figure 4.1). They were caught using a combination of monofilament gillnets and demersal longlines (detailed in chapter 3) and a subsample of individuals (about 25 per sampling event) were frozen and stored until transport to the laboratory for biological examination.

In the laboratory, specimens were thawed, sexed and total length (TL, measured from the tip of the rostrum to the end of the extended upper caudal lobe to nearest mm) and total body weight

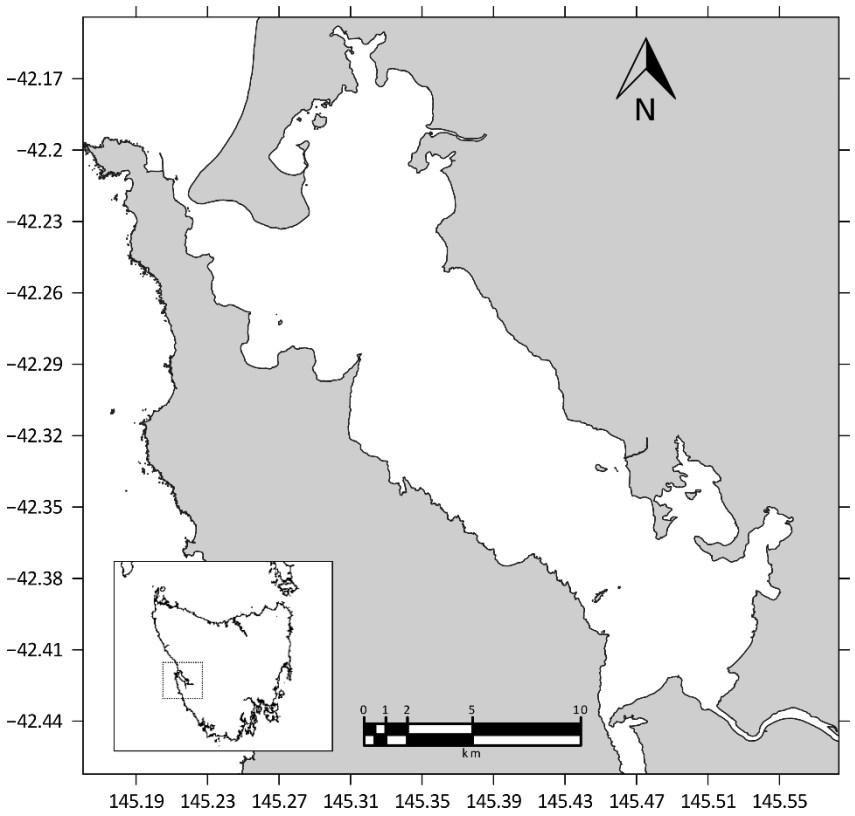

Figure 4.1. Map of Macquarie Harbour in western Tasmania where *S. acanthias* were sampled.

(TW, to nearest 0.1 g) recorded. The second dorsal spine and 5–10 post-cranial vertebrae were removed and frozen until further processing.

4.2.2. Preparation of calcified structures for ageing

Second dorsal spines were cleaned of soft tissue using a scalpel blade and air dried before ageing. Broken and incomplete spines were excluded from the ageing sample. Vertebrae were sampled from the same region in all individuals (i.e. cervical region) to ensure consistency. The use of adjacent vertebrae has been demonstrated to have no effect on growth ring counts when vertebrae are taken from the same spinal region (Piercy et al., 2006).

Soft tissue was carefully removed from the vertebrae with a scalpel blade before embedding in epoxy resin. Vertebrae were sectioned (~400 µm) through the focus on the sagittal plane using a high-speed saw (Gemmasta Deluxe GS6D) with a single diamond plated blade. The vertebral sections were placed into tissue cassettes and decalcified with trical-decalcifying fluid (CH_2O_2 7.6%, HCl 1.6%, Australian Biostain) under agitation for a minimum of 1 hr or until the tissue

Table 4.1. Harris haematoxylin staining procedure for *S. acanthias* vertebrae, from Bubley et al. (2012). Modified from Nathanson et al. (2007)

Time (min)		Description
Decalcification		
5	100% dH2O rinse	
60-180	Trical-Decalcifyer	Use agitation. Adjust time until sample is fully decalcified. Lower solution of decalcifier can be used to slow down the process and control progress more accurately
60	Running water	
Histological stain procedure		
5	100% dH2O rinse	
10	Harris haematoxylin	Times can be adjusted depending on the strenght of the stain to enssure proper staining
Until clear	Running water	
2	Acid alcohol	Adjust time for proper de-staining. Acid alcohol consisted of 99% ethanol solution (65% dH2O, 35% absolute ethanol) and
10	Running water	
2	100% dH2O rinse	
10	25% glycerine	
10	50% glycerine	
10	75% glycerine	
10	100% glycerine	At this point samples are ready for mounting and storage

was fully decalcified. Samples were then rinsed in running water for 1 hr to stop the decalcification process. Sectioned vertebrae were then stained using the modified Harris haematoxylin procedure outlined in Bubley et al. (2012) (Table 4.1) and mounted onto glass microscope slides using glycerol jelly.

4.2.3. Age determination

Stained vertebrae were photographed with a camera mounted stereo-microscope (Leica MZ7.5). Magnification was adjusted based on the size of the sampled vertebra and a standardized scalebar was digitally appended to every individual image capture to allow precise measurements to be made from the images. When necessary, images were digitally edited to enhance contrast and improve readability (as per Campana, 2014). Vertebral CR was defined as the distance from the focus to the edge along the corpus calcareum (Figure 4.2) and was measured on the internal face of a thin section along the sagittal plane to the nearest μm.

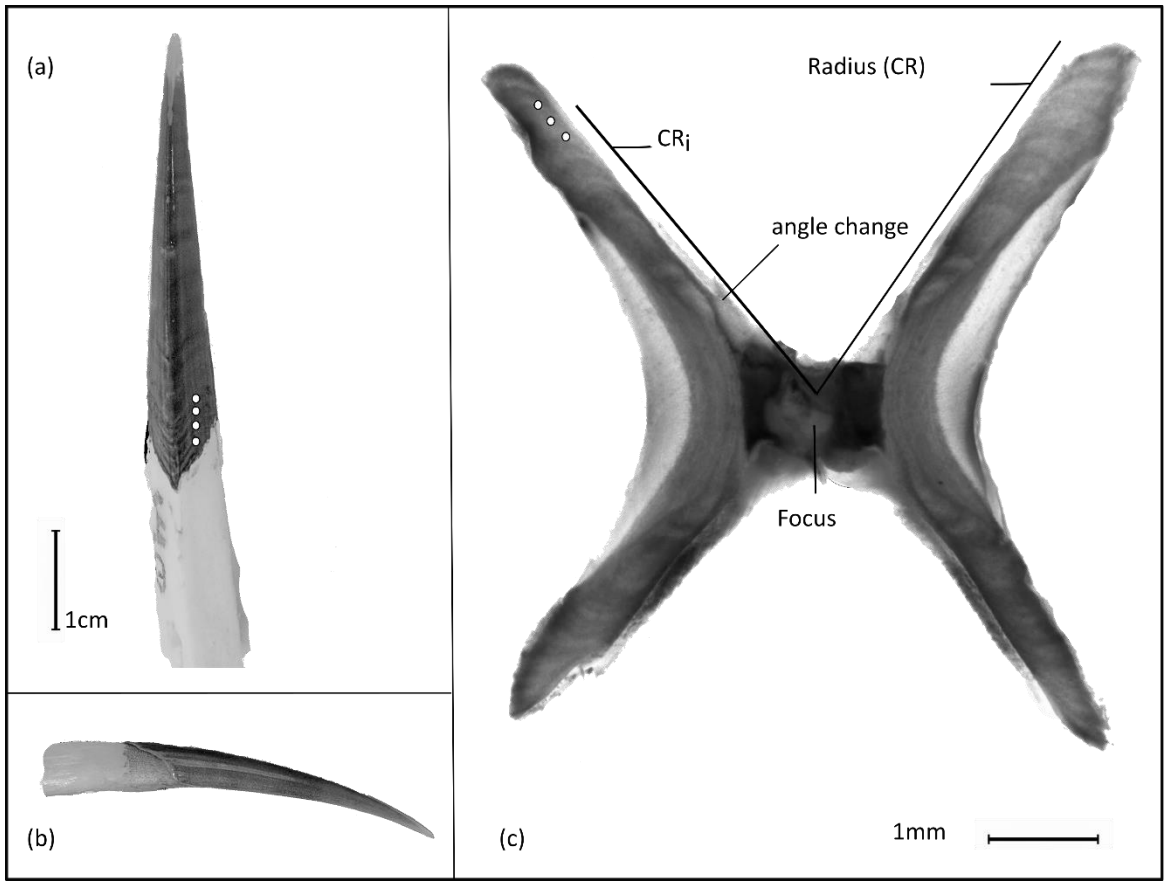

Figure 4.2. Hard-structures used for age determination in *S. acanthias*. **(a)** Second dorsal spine showing example of individual growth increments (○) and **(b)** example of hard to age dorsal spine with poor definition between growth increments. **(c)** Stained vertebral cross-section showing example of individual growth increments (○). Total radial length (CR) and radial length to age i (CR_i) measured from the focus along the corpus calcareum

To confirm the adequacy of using vertebrae to estimate age and growth, the relationship between CR and TL was explored. Selection of an appropriate method for age back calculation (Section 4.2.4) requires knowledge of the shape of the relationship between these two variables, which may take a linear or quadratic form. Both models were fitted to males and females separately, model fit was compared using ANOVA and the adjusted coefficient of determination was used to evaluate fit.

Age determination from vertebrae was based on the methodology described by Bubley et al. (2012) and involved counting increments along the corpus calcareum of the stained thin sections. Unstained, whole spines were aged following Ketchen (1975) by counting the growth increments in the outer enamel layer of the spine. For both structures, each year or annulus

consisted of a dark and clear section. A subjective readability score (RI) was assigned on a sliding scale, where 1 (unambiguous sample), 2 (less clear than 1, non-consecutive repeat readings by the primary reader were within 3% of one another other) and 3 (unreadable sample, large ambiguous sections). If adjacent annuli could not be distinguished with certainty, the section was only counted as one year.

The primary reader aged all samples of both structures on two separate occasions while the secondary reader aged a subset of 50 vertebrae. Ages were determined without knowledge of the size (from an image file without a scalebar) and sex of the individuals. Inter- and intra-reader precision was compared using average percentage error (APE) and uncertainty around the estimate was calculated using a bootstrapping procedure (n=10000). Systematic bias was tested by comparing the bootstrapped mean APE to a bias-corrected mean APE and through a bias plot (Figure 4.4) (Beamish and Fournier, 1981).

While age validation was not achieved in this study, the annual deposition of growth increments in the spine of *S. acanthias* has been validated in other populations using tetracycline (Tucker, 1985), X-ray spectroscopy (Jones and Geen, 1977) and bomb radiocarbon dating (Campana et al., 2006). Age estimates obtained from vertebrae have also been verified with marginal increment analysis (Bubley et al., 2012). We assumed similar annual periodicity in both ageing structures with age in years based on the number of complete (opaque and translucent)

increments counted.

4.2.4. Back calculation

Back calculation was implemented using the vertebrae and used to estimate TL at past ages (L_i) for each individual. This approach can help compensate for small sample sizes or missing age classes (Cailliet et al., 2006). These techniques assume that the proportional changes in radii (CR_i) between consecutive increments in the vertebrae are proportional to the growth in length of the individual during that period. The size at capture is then used to infer the length of the

individual at each previous year (Figure 4.2). Image analysis software ImageJ (Schindelin et al., 2015) was used to measure the distance in a straight line from the focus to the edge of the opaque portion of each growth increment to the nearest μm.

Two back calculation methods commonly used in elasmobranch studies were tested; the modified Fraser-Lee (Campana, 1990) and modified Dahl-Lea (Francis, 1990) methods. The modified length at birth Fraser-Lee method, which assumes a linear relationship between the ageing structure radius and TL has the advantage of incorporating additional exogenous information in the form of size at birth, and is described as:

$$L_i = L_c + [(CR_i - CR_c)(L_c - L_{Birth})/(CR_c - CR_{Birth})]$$

where L_i, L_c, and L_{birth} = total lengths at age i, capture and birth, respectively; CR_i, CR_c, and CR_{birth} = vertebra centrum radii at age i, capture and birth respectively.

The second method was the quadratic modified Dahl-Lea, which can be applied when the relationship between ageing structure diameter and TL is best described by a quadratic function:

$$L_i = L_c\left[\left(a + bCR_i + cCR_i^2\right)/\left(a + bCR_c + cCR_c^2\right)\right]$$

where a, b and c are the quadratic fit parameters.

4.2.5. Length at age modelling

A total of six growth models were fitted to the age and growth data calculated using vertebrae (see section 4.3.2) for males and females separately: 1) the von-Bertalanffy growth model (VBGM), 2) the two-parameter reformulation of the VBGM model with a fixed L_o (VBGM L_0), 3) the Gompertz growth model (GGM), 4) a logistic model (LM), 5) the hyper-k two phase VBGM (TPVB hyper-k) and 6) the log-L_∞ VBGM (TPVB log-L_∞) (Table 4.2). In addition to modelling the observed data, models were also fitted to the back calculated (i.e. observed + back calculated size at age data for all individuals) and mean back calculated data (i.e. observed + mean size per

year estimated from back calculated data) (as per Tribuzio et al., 2010). The models were fitted by nonlinear least-squares regression using R environment (R Core Team, 2013). Confidence intervals for the estimated parameters were calculated by a bootstrap procedure (n=5000).

The fixed term L_0 was assumed to be the mean size at birth (Campana et al., 2009). The biphasic models (i.e. TPVB; Table 4.2) simulate the energy allocated to reproduction by a smooth transition between two values (phases) of a model parameter (k and L_∞ respectively) (Minte-Vera et al., 2016). These two models assume that the shift between phases takes a logistic shape with the inflection point around the size at maturity, which is modelled using a logistic maturity ogive. The biphasic models (i.e. hyper-k and log-L_∞) were initially fitted using a calculated inflection point but convergence could not be achieved. As an alternative, fixed values were used based on observed size at maturity data (TL_{50}) (Chapter 3).

Newly born individuals were absent from the sample, therefore size at birth could not be directly established. For calculations requiring a fixed size at birth, the length reported in the North Atlantic (250 mm) was used (Bubley et al., 2012), as the size of the few near-term embryos

Table 4.2. Models included for multi model inference, where L_t is Total length at age t (mm), L_∞ represent asymptotic length (mm), k is a growth coefficient (yr^{-1}) and t_0 is the theoretical age at size 0 (yr.). In the logistic model, a represents the inflection point of the curve. For the two-phase variants of the VBGM, h is a modifier of k and represents the magnitude of the maximum difference with the standard model; t_{50} and t_{95} are the age at 50% and 95% maturity

Model		ϕ	Model	Source
m1	VBGM	ϕ= 3	$L_t = L_\infty - (L_\infty - L_0)exp^{-kt}$	von Bertalanffy (1938)
m2	VBGM, L_0=250	ϕ= 2	$L_t = L_\infty - (L_\infty - L_0)exp^{-kt}, \quad L_0 = 250mm$	Fabens (1965)
m3	Gompertz	ϕ= 3	$L_t = L_0 exp^{G(1-exp^{-kt})}, \quad G = \ln\frac{L_\infty}{L_0}$	Mollet et al. (2002)
m4	Logistic	ϕ= 3	$L_t = \frac{L_\infty}{1 + exp^{-k(t-a)}}$	Ricker (1979)
m5	T-P VB hyper-k	ϕ= 5	$L_t = L_\infty\{1 - exp^{[-k[1-h((t-t_h)^2+1)^{-1}](t-t_0)]}\}$	Soriano et al. (1992)
m6	T-P VB log-L_∞	ϕ= 5	$L_t = \left\{L_{1,\infty} + (L_{2,\infty} - L_{1,\infty})\left[1 + exp^{\left(\frac{(-\ln(19))(t-t_{50})}{t_{95}-t_{50}}\right)}\right]^{-1}\right\}\{1 - exp^{-k(t-t_0)}\}$, where $L_{2,\infty} = L_{1,\infty} + \delta L_\infty$	Minte-Vera et al. (2016)

recorded in Macquarie Harbour was close to this value (n=3, 219 mm ±4.72). The biphasic models (i.e. hyper-k and log-L_∞) were initially fitted using a calculated inflection point but convergence could not be achieved. As an alternative, the inflection point was treated as a fixed factor based on size at 50% maturity estimated using the maturity ogives in Chapter 3 for males (t_{50}= 519 mm) and females (t_{50}= 559 mm).

Models were evaluated using a MMI theoretic approach based on a small sample corrected Akaike's information criterion (AICc) (as per Katsanevakis and Maravelias, 2008). The AICc (Hurvich and Tsai, 1989) modifies the standard AIC (Akaike, 1973) by considering the sample size and number of variables and was selected over AIC due to its improved performance when sample sizes are <200 (Zhu et al., 2009). AICc was estimated as:

$$AIC_c = AIC + \frac{2\emptyset\ (\emptyset + 1)}{n - \emptyset - 1}$$

where for least squares AIC= n log(σ^2) + 2ϕ, σ^2= RSS/n, n=sample size and ϕ=total number of regression parameters estimated including the variance (σ^2). Normal deviations with constant variance were assumed. The model with the lowest AICc value (AIC_{min}) was considered the 'best' among the candidate models and the remaining models were ranked based on the AICc differences (Δ_i= $AICc_i$ – AIC_{min}).

Using the calculated AICc values, a weighted relative probability was calculated for each model. The Akaike weight (w_i) acts as a Bayesian a posterior model probability for the candidate models (Burnham and Anderson et al., 2004) representing the true fit for a given dataset. The threshold for selecting single "best" model was $w_i \geq 0.9$ (as per Katsanevakis and Maravelias, 2008). Akaike weights are calculated as:

$$w_i = \frac{\exp(-\Delta_i/2)}{\sum_1^{\emptyset-1} \exp(-\Delta_i/2)}$$

Model averaged estimates of the coefficients L_0 and L_∞ were estimated as a weighted average using Akaike weights as a scaling factor (as per Katsanevakis, 2006). For L_o rescaled Akaike

weights ignoring the 2 parameter VBGM, which had a fixed value for L_0 (Katsanevakis, 2006 and Maravelias, 2008). The growth coefficient of the different models (k) was not directly comparable, so the derivative form of each model was used to calculate instantaneous growth rates at birth (dL/dt_0), age at 50% maturity (dL/dt_{50}) and age at 95% of the asymptotic length ($dL/dt_{L\infty,0.95}$) as per Thorson and Simpfendorfer (2009).

Knowledge of female longevity is essential for demographic analysis (Cortes, 2002), but due to the lower frequency of individuals at extreme sizes, maximum ages may be underestimated. Therefore, theoretical maximum age was calculated from the growth models as age at 99% of L_∞ (Skomal and Natanson, 2003) and as $t_{max}=7\ln(2/k)$ (Mollet et al., 2002).

4.3. RESULTS

4.3.1. Age determination

A total of 154 individuals were sampled (Male= 66, Female=88). Total length (TL) ranged from 209 mm (near term embryo) to 980 mm. Smaller size classes (250–550 mm TL) were mostly absent from the sample, with only 8 individuals in the sample (including 3 near term embryos). Vertebrae were collected from all individuals, whereas the dorsal spine of 28 individuals showed extreme wear or breakage and could not be used for age determination (Male=15, Female=13).

Most spines (86.2 %) contained portions in which the age increments were harder to interpret than in the rest of the spine. Age increments in spines obtained from Tasmanian populations seem to have better defined and easier to interpret results (personal observation), but further sampling is needed to make an appropriate comparison, In samples from Macquarie Harbour, there were no apparent patterns regarding the width or location along the spine of the harder to read portions, and they occurred regardless of sex or size of the individual. . This greatly affected the readability of the spines, and only 13.7% of spines were given an RI= 1, 33.9 % had RI=2 and 52.4% had RI=3. Despite this, age estimates from all unworn spines were retained, but

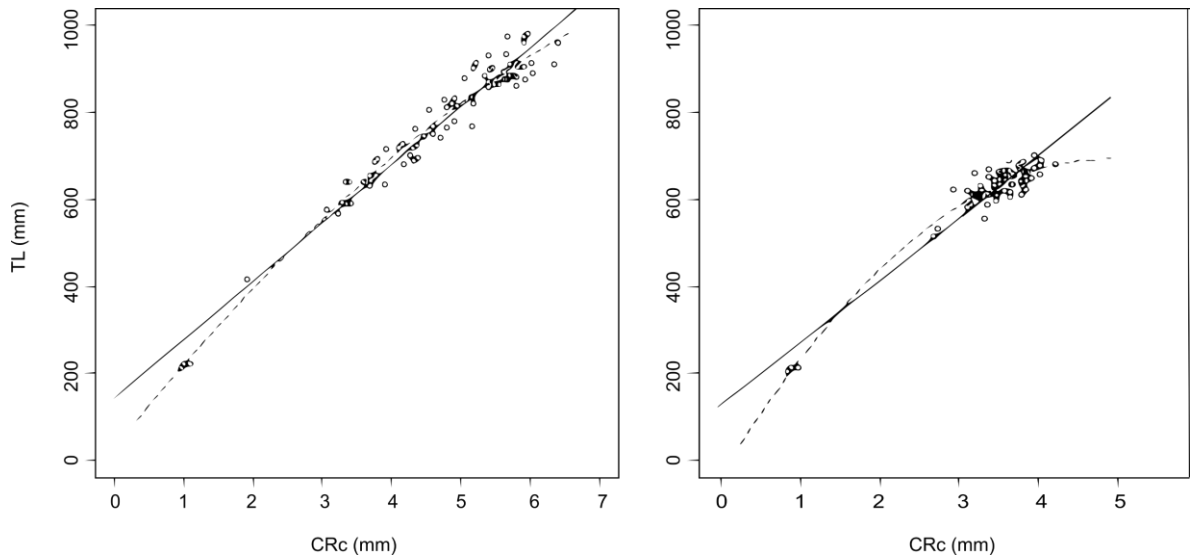

Figure 4.3. Relationship between vertebrae radius at capture (CRc) and total length female (a) and male (b) *S. acanthias,* showing a linear (—) and quadratic fit (---). CRc was measured from the focus to the edge of the corpus calcareum in a thin section taken along the sagittal plane of each vertebrae. Vertebrae from pre-natal near-term embryos of both sexes were included to provide better estimates of smaller expected TLs of younger fish. Parameters for the quadratic model for males were (a=-53.3, b=296.7, c=-29.5) and for females (a=27.5, b=200.5, c=-8.3)

Table 4.3. Comparison of linear and quadratic curves for describing the relationship between vertebrae radius (CR) and total length (TL) for male and female *S. acanthias.* RSE= residual standard error, RSS=residual sum of squares (ANOVA)

		Fit			Performance	
		RSE	F	p	adj R^2	RSS
Female						
	Linear	36.25	1477	<0.001	0.95	99871
	Quadratic	32.18	948	<0.001	0.96	77653
Male						
	Linear	31.96	499	<0.001	0.89	61291
	Quadratic	24.45	448	<0.001	0.93	35258

in those with difficult to interpret sections, adjacent increments that could not be identified as distinct annuli were counted as a single year.

Stained vertebrae were easier to read than spines, and only 15.2 % of the sample was given an RI=1, 41.3 had RI=2 and 43.5 % RI=3. Vertebrae centrum radius was positively correlated with length for both sexes (Figure 4.3) with a quadratic function providing a better fit than the linear model in females and both models providing a similar fit in males (Table 4.3). Three near-full-term embryos (i.e. no external yolk) were used to estimate the position of the birth increment in vertebrae. Distance from the embryo vertebral focus to the edge of the corpus calcareous was ~1.3 mm. This was consistent with the location of a slight angle change, and the first opaque

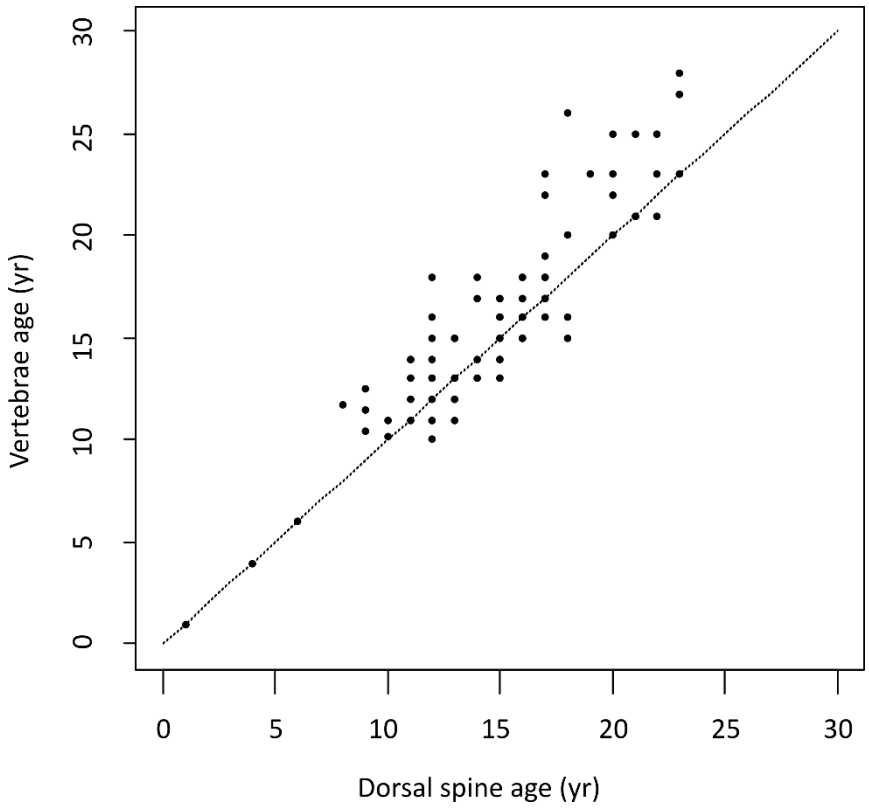

Figure 4.4. Bias plot for estimated ages of *S. acanthias* from vertebrae and dorsal spines, showing a 1:1 relationship (–).

Table 4.4. Comparison between ageing structures (i.e. vertebrae v spines) and inter-and intra-reader precision estimates for *S. acanthias* ages obtained from stained vertebrae. Average percentage error (A.P.E.) from mean bootstrapped estimates and A.P.E. corrected for bias (5000 rep). 95% confidence intervals for A.P.E. calculated from bootstrapped error.

	N	A.P.E.	Bias corrected	s.e.	95% c.i.
Structure (vertebrae / spine)	100	7.35	7.4	0.6	6.1-8.6
Intra-reader variation	141	4.94	4.92	0.3	4.2-5.6
Inter-reader variation	50	5.45	5.45	0.3	4.7-6.1

increment, suggesting this represented a 'birth increment' (Figure 4.2) and was subsequently considered to represent age=0 during ageing.

4.3.2. Precision

Age estimates obtained using spines were lower than those obtained from vertebrae, particularly for larger, older individuals (Figure 4.4). The directional bias in structure-specific age estimate occurred because older increments (early life) were often difficult to interpret due

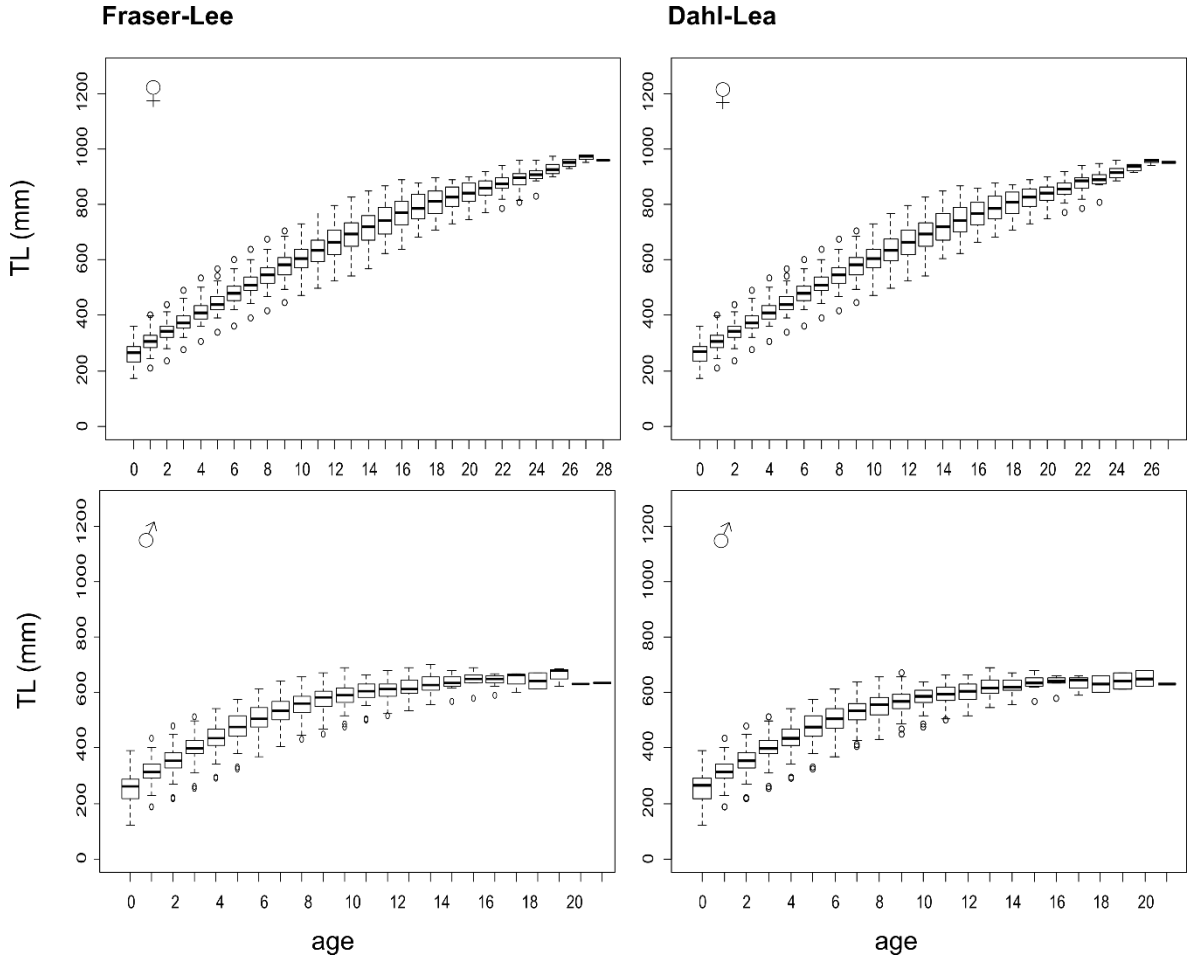

Figure 4.5. Median (black line), interquartile range (box) and 90% confidence interval (whiskers) of back calculated estimates of length (TL), estimated using two models, and age (yr.), estimated using vertebrae, of male and female *S. acanthias*.

to wear (Figure 4.2). Thus, age estimated from vertebrae rather than spines was used for growth modelling.

Precision of vertebrae age for intra-reader and inter-reader variation were 4.9%, and 5.4% respectively, as determined by mean APE (Table 4.4); comparing APE to bias corrected APE showed no systematic bias for either (Table 4.4). Inter- and intra-reader differences in estimated age from vertebrae were within the range of what is commonly considered acceptable in ageing studies (Goldman, 2005).

4.3.3. Age back calculation

Both back calculation methods produced similar results (Figure 4.5). The modified Dahl-Lea method produced realistic sizes at age=0 (266 ±36 mm) despite not incorporating an explicit

estimate of observed size at birth such as included in the back-calculation equation for the Fraser-Lee method. The estimated size at age data produced by the Dahl-Lea model were selected because the underlying quadratic function described the relationship between CR and TL better than the linear function assumed by the Fraser-Lee method in females ($X^2_{(1)}$=19.63, p<0.001) and males ($X^2_{(1)}$=34.28, p<0.001) (Table 4.3).

4.3.4. Age and growth analysis

Age estimates ranged from 0–28 years (based on vertebrae). Males younger than 6 years and females younger than 8 years were poorly represented in the aged sample. Maximum observed ages were 22 and 28 years for males and females respectively.

Male and female growth was sexually dimorphic (Table 4.5 and 4.6). Females grew to larger size and matured later than males based on the age at which TL_{50} (Chapter 3) was achieved (males: 519 mm at 4–6 years and females: 559 mm at 8–9 years). Male growth was asymptotic, and some of the individuals in the sample were larger that the estimated L_∞ from all models. In contrast, female lengths at age were slower to approach their asymptote, and none of the individuals reached sizes approaching L_∞. Theoretic estimates of longevity were lower when calculated as 99% of L_∞ than when derived from the growth coefficient k (7ln(2)/k), however in both cases estimated longevity was much larger than the observed maximum age (Table 4.6).

None of the six growth models consistently outperformed the others across all scenarios (observed, back calculated and mean back calculated data) (Table 4.5 & 4.6). Model ranking based on AIC_c was consistent with residual errors (RSE) and standard AIC (Table 4.5 & 4.6). This supports the adequacy of using w_i as the single criteria for model ranking. Akaike weights of all models were >0.9, thus no single candidate model should be singularly chosen in any of the scenarios.

Table 4.5. Growth parameters of female *S. acanthias* aged using vertebrae. Models fitted to observed, back calculated and mean back calculated data. Models 1-6 (Table 4.1) ranked based on performance using the corrected Akaike information criteria (AIC_c) and Akaike weights as a % ($100*w_i$). Parameter estimates and standard error for 2, 3 and 4 parameter models are provided. Model averaged results were calculated as a weighted mean based on w_i. Instantaneous growth rate (mm/yr.) at birth, 50% maturity and 95% max length were calculated using the derivative of each model. Age at 50% maturity and two theoretic estimates of longevity are also provided.

			Model fit				L_∞	se(±)	L_0	se(±)	k	se(±)	ϕ4	se(±)	dL	dL	dL	t50	Longevity		
Model		Rank	R.S.E.	AICc	Δ_i	w_i	(mm)		(mm)		(y^{-1})				$dt_{(birth)}$	$dt_{(95)}$	$dt_{(mat50)}$	(yr)	7ln(2)/k	L_{99}	Observed
Observed data (n=88)																					
m2	VBGM, L0=250	1	62.62	858.88	0.00	24.43	1302.24	153.79	250.00	0.00	0.042	0.008			43.8	2.7	30.9	8	52.1	45.8	28
m3	Gompertz	2	61.87	859.26	0.38	20.19	1059.63	57.11	223.71	59.42	0.098	0.012			14.8	2.2	15.2	9	22.2	19.4	
m4	Logistic	3	61.89	859.33	0.45	19.53	990.86	33.63	234.56	30.34	0.151	0.016			27.0	7.1	36.7	9	14.4	12.5	
m1	VBGM	4	62.19	860.06	1.17	14.29	1242.78	123.38	215.94	32.49	0.049	0.009			49.8	3.0	33.2	8	44.8	39.5	
m5	TPVB hyper-k	5	62.09	861.06	2.17	13.58	1165.68	123.61	220.66	33.86	0.056	0.013	0.96	0.81	52.9	3.3	33.9	9	38.8	34.1	
m6	TPVB log-Linf	6	62.26	861.48	2.60	7.98	1149.85	171.07	211.10	33.71	0.062	0.016	1253.24	132.57	64.4	3.6	36.5	8	35.1	30.9	
Model-averaged							1153.24	103.74	229.72	29.85					38.4	3.6	30.1		34.5	30.3	28.0
Backcalculated data																					
m3	Gompertz	1	47.85	14924.85	0.00	59.51	1060.68	15.07	273.61	1.97	0.090	0.002			14.4	2.0	13.9	8	24.2	20.9	28
m6	TPVB log-Linf	2	47.86	14926.29	1.44	28.97	1350.61	47.05	265.75	3.82	0.038	0.002	1336.93	46.47	40.2	2.5	29.7	8	57.8	50.7	
m1	VBGM	3	47.92	14928.86	4.01	8.02	1350.43	41.53	262.14	3.37	0.039	0.002			41.9	2.6	30.5	8	56.4	49.5	
m5	TPVB hyper-k	4	47.93	14930.54	5.69	3.46	1336.90	51.02	261.87	3.37	0.039	0.003	0.014	0.025	42.2	2.6	30.5	8	55.3	48.5	
m2	VBGM, L0=250	5	48.11	14939.51	14.66	0.04	1273.14	27.43	250.00	0.00	0.044	0.002			44.6	2.8	31.1	8	49.8	43.7	
m4	Logistic	6	48.27	14949.60	24.75	0.00	969.25	9.48	283.87	2.75	0.141	0.003			28.2	6.5	33.3	8	15.4	13.1	
Model-averaged							1177.55	27.71	270.00	2.67					25.1	2.2	20.4		37.6	32.8	28.0
Mean backcalculated data																					
m2	VBGM, L0=250	1	53.18	1136.66	0.00	44.88	1293.10	89.14	250.00	0.00	0.042	0.005			44.1	2.7	31.0	8	51.3	45.1	28
m1	VBGM	2	53.35	1138.48	1.82	18.05	1263.78	111.35	238.10	20.84	0.045	0.008			46.0	2.8	31.6	8	48.4	42.5	
m3	Gompertz	3	53.40	1138.69	2.03	16.25	1075.29	42.44	254.48	40.44	0.091	0.008			14.4	2.1	14.4	8	24.0	20.8	
m6	TPVB log-Linf	4	53.49	1140.22	3.56	7.57	1229.74	95.99	231.86	21.47	0.049	0.009	1258.76	109.40	50.6	3.0	33.1	8	44.0	38.7	
m5	TPVB hyper-k	5	53.59	1140.59	3.93	6.28	1253.67	105.56	238.78	19.52	0.046	0.007	0.055	0.116	46.3	2.9	31.7	8	47.6	41.8	
m4	Logistic	6	53.84	1140.38	3.73	6.96	1005.41	26.94	269.27	13.43	0.137	0.010			26.9	6.5	33.9	9	15.9	13.6	
Model-averaged							1224.99	82.77	247.82	14.12					39.1	2.9	28.8		43.1	37.8	28

R.S.E.= residual standard error

s.e. = standard error for the parameter estimates

ϕ4= additional parameter for bi-phasic models. M5= h, M6=Linf2

In model 5, the growth coefficient (k) is modified by h at a scale derived by A. A is a derivative of time around the inflection point at time of maturity. Therfore at L50, At=1 and growth=k*h

In model 6 the phase specific values of Linf were used to estimate the growth rate at t0, L50 and t95

Table 4.6. Growth parameters of male *S. acanthias* aged using vertebrae. Models fitted to observed, back calculated and mean back calculated data. Models 1-6 (Table 4.1) ranked based on performance using the corrected Akaike information criteria (AIC_c) and Akaike weights (w_i). Parameter estimates and standard error for 2, 3 and 4 parameter models are provided. Model averaged results were calculated as a weighted mean based on w_i. Instantaneous growth rate (mm/yr.) at birth, 50% maturity and 95% max length were calculated using the derivative of each model.

			Model fit				L_∞	se(±)	L_0	se(±)	k	se(±)	ϕ4	se(±)	dL	dL	dL	t50	Longevity
Model		Rank	R.S.E.	AICc	Δ_i	w_i	(mm)		(mm)		(y^{-1})				$dt_{(birth)}$	$dt_{(95)}$	$dt_{(mat50)}$		Observed
Observed data (n=66)																			
m5	TPVB hyper-k	1	31.68	621.13	0.00	23.54	648.09	7.48	219.58	17.94	0.348	0.074	1.625	0.619	149.0	11.3	31.0	4	22
m4	Logistic	2	32.04	621.25	0.13	22.10	650.12	6.45	218.46	17.95	0.411	0.043			59.6	12.7	32.2	4	
m3	Gompertz	3	32.11	621.51	0.39	19.39	653.37	7.84	218.48	8.56	0.333	0.041			34.6	4.6	12.6	4	
m1	VBGM	4	32.19	621.85	0.72	16.42	657.15	9.03	218.76	18.13	0.268	0.037			117.3	8.8	26.3	4	
m2	VBGM, L0=250	5	32.67	622.47	1.34	12.03	657.97	9.99	250.00	0.00	0.257	0.038			104.8	8.5	25.4	4	
m6	TPVB log-Linf	6	32.33	623.70	2.57	6.51	649.84	16.51	219.20	17.91	0.413	0.162	1454.46	32.66	178.1	13.4	37.6	4	
Model-averaged							652.29	8.46	222.60	13.99					98.4	9.7	26.7		22
Backcalculated data																			
m3	Gompertz	1	46.79	8554.66	0.00	56.05	662.65	6.98	258.02	3.55	0.208	0.008			22.0	2.9	8.6	6	22
m2	VBGM, L0=250	2	46.88	8556.88	2.22	18.46	690.53	9.03	250.00	0.00	0.144	0.006			63.3	5.0	18.9	6	
m1	VBGM	3	46.91	8558.82	4.16	7.01	691.69	10.22	251.31	4.57	0.143	0.007			62.8	4.9	18.9	6	
m6	TPVB log-Linf	4	46.88	8558.86	4.20	6.86	689.83	14.62	254.26	4.99	0.140	0.008	677.41	10.22	59.4	4.8	18.4	6	
m5	TPVB hyper-k	5	46.88	8559.06	4.40	6.21	683.78	11.08	251.13	4.74	0.150	0.009	0.069	0.049	65.0	5.1	18.7	6	
m4	Logistic	6	46.92	8559.34	4.68	5.41	646.38	6.05	264.40	3.82	0.275	0.010			43.0	8.4	20.8	6	
Model-averaged							672.13	8.31	255.73	3.15					38.9	4.0	13.2		22
Mean backcalculated data																			
m4	Logistic	1	31.28	831.96	0.00	58.75	650.85	5.84	230.68	11.87	0.359	0.025			53.5	11.1	28.3	5	22
m5	TPVB hyper-k	2	31.48	834.25	2.29	18.69	656.98	7.63	223.25	13.98	0.245	0.024	0.431	0.146	106.3	8.1	24.0	5	
m3	Gompertz	3	31.71	834.29	2.33	18.34	656.56	6.59	224.52	3.27	0.285	0.020			29.8	4.0	11.1	5	
m6	TPVB log-Linf	4	32.34	838.83	6.87	1.89	663.31	9.87	226.16	16.95	0.207	0.024	606.69	37.44	78.8	6.9	21.6	4	
m1	VBGM	5	32.61	839.04	7.08	1.71	665.11	8.79	219.67	14.29	0.216	0.019			96.3	7.2	22.9	5	
m2	VBGM, L0=250	6	33.23	841.10	9.13	0.61	668.32	8.70	250.00	0.00	0.202	0.017			84.4	6.7	22.1	4	
Model-averaged							653.56	6.45	227.98	10.75					60.4	9.1	24.1		22

R.S.E.= residual standard error

s.e. = standard error for the parameter estimates

ϕ4= additional parameter for bi-phasic models. M5= h, M6=Linf2

In model 5, the growth coefficient (k) is modified by h at a scale derived by A. A is a derivative of time around the inflection point at time of maturity. Therfore at L50, At=1 and growth=k*h

In model 6 the phase specific values of Linf were used to estimate the growth rate at t0, L50 and t95

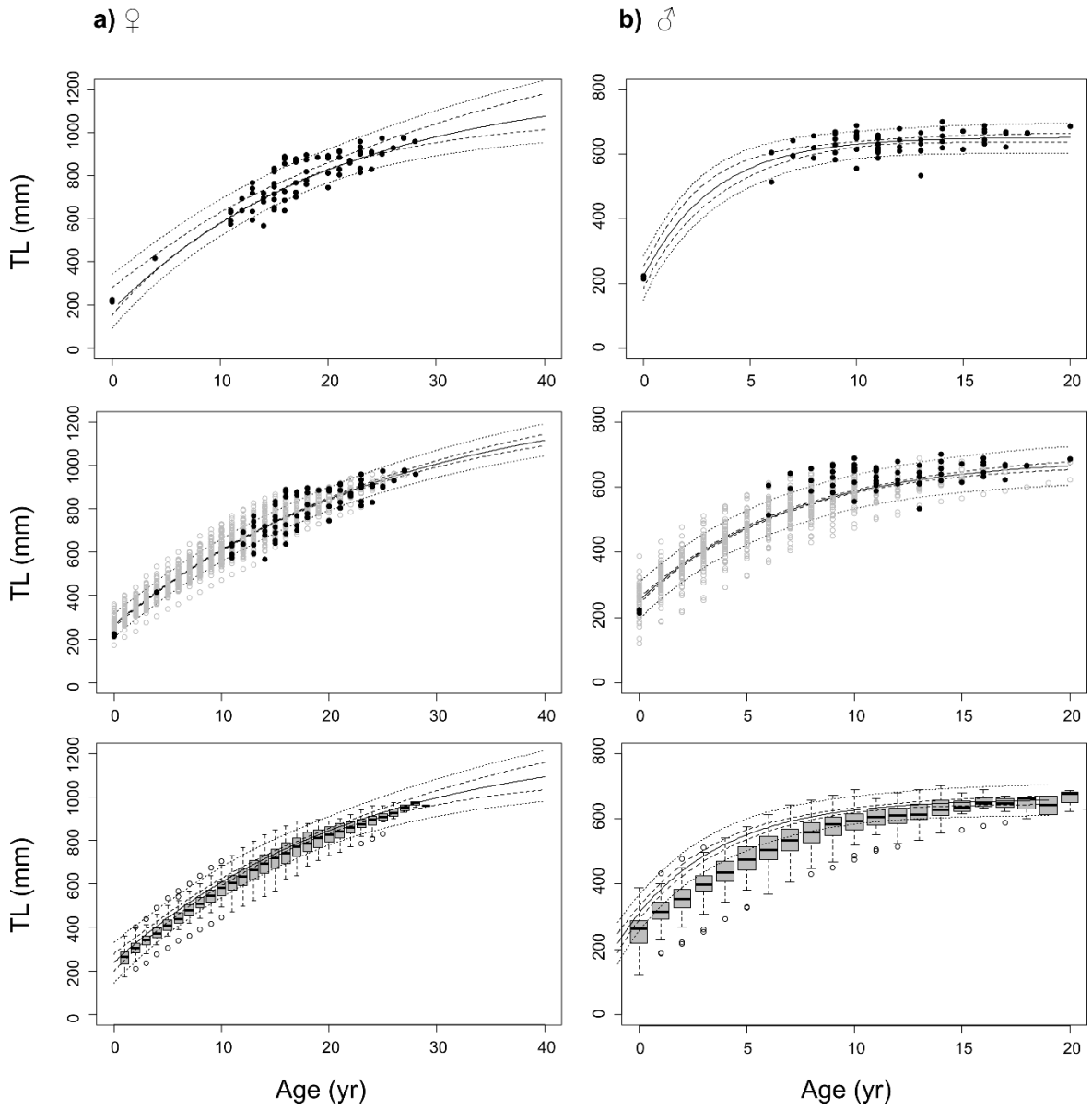

Figure 4.6. Estimated mean growth for observed data (●) (top row), back calculated data (○) (middle row) and mean back calculated data (bottom row) of female **(a)** and male **(b)** *S. acanthias* in Macquarie Harbour based on vertebral age estimates. Model shown is the best ranked (Tables 4.5 and 4.6) model (—), with confidence intervals (- - -) and prediction intervals (⋯) calculated by bootstrapping (n=5000). Boxplots represent median (black line), interquartile range (box) and 90% confidence interval (whiskers) of back calculated data.

The estimates of L_∞ produced by the Gompertz and logistic models were generally the smallest of the models. For the observed and mean back calculated datasets, models were weighted similarly, as evidenced by the small differences in w_i. In contrast, when the back calculated data were included, model type more strongly influenced goodness of fit, with the Gompertz and standard VBGM performing best. The logistic, Gompertz and model-averaged parameters had the lowest error regardless of sex or dataset (Table 4.7). Prior studies have typically used a two

Table 4.7. Accuracy in parameter estimation (% s.e.) of the different growth models and the model averaged estimates. Accuracy calculated as the average proportional error for each estimate from the three datasets in each sex. Total error is the mean uncertainty around all parameters for each model.

		L_∞	L_0	k	Total error
Females					
m1	VBGM	7.3	8.4	13.62	9.75
m2	VBGM, L0=250	7.0		11.84	9.39
m3	Gompertz	3.6	14.4	8.01	8.66
m4	Logistic	2.4	6.3	6.58	5.08
m5	TPVB hyper-k	7.6	8.3	15.05	10.31
m6	TPVB log-Linf	8.7	8.9	16.76	11.46
MMI		6.0	6.6	10.10	7.56
Males					
m1	VBGM	1.4	5.5	9.25	5.39
m2	VBGM, L0=250	1.4		9.20	5.29
m3	Gompertz	1.1	2.3	7.76	3.70
m4	Logistic	0.9	4.9	6.91	4.26
m5	TPVB hyper-k	1.3	5.4	12.48	6.41
m6	TPVB log-Linf	2.0	5.9	18.71	8.88
MMI		1.2	4.1	9.38	4.88

or three parameter version of the VBGM to model the growth of *S. acanthias*, so to facilitate comparison between *S. acanthias* in Macquarie Harbour and published values for other populations, results from the standard VBGM are provided in Table 4.8.

4.4. DISCUSSION

The present study provides the first quantitative assessment of the age and growth of *S. acanthias* in any Australian population. Age estimates in this study ranged from 0-28 yrs., however, females < 10 years and males < 5 years were poorly represented in the sample. Despite this, the growth characteristics of the population were largely consistent with what is known about the species. Using stained vertebrae reduced the likelihood of underestimating age in larger individuals, substantiating the preferred use of vertebrae over the typical use of dorsal spines when ageing *S. acanthias*. In contrast, for *S. suckleyi*, which is reported to reach ages of up to 107 yrs. despite having similar sizes to *S. acanthias*, the method for ageing vertebrae was not successful (Tribuzio et al., 2017). It is possible that the increased age of *S. suckleyi* will result

Table 4.8. Comparison of published female *S. acanthias* growth (VBGM), longevity and maturity from other populations.

Location	Age and growth					Maturity		Source	
	t_0 (yr^{-1})	L_0 (mm)	L_∞ (mm)	k (yr^{-1})	t_{max} (yr)	t_{50} (yr)	L_{50} (mm)		
Mediterranean and Black Sea									
SE Black Sea	-0.73		1450	0.17	14	5	880	Avsar (2001)	
SE Black Sea	-1.70		1370	0.13		12	722	Demirhan et al. (2007)	
Eastern Mediterranean							518 *	Chatzispyrou (2005)	
Atlantic Ocean									
NE Atlantic (Ireland)		133	988	0.09				Fahy (1989)	
NE Atlantic (Ireland)		246	1120	0.15	22	15	785	Henderson (2002)	
North Sea		307	1371	0.05				Sosinski (1978)	in Avsar (2001
North sea (Britain)		332	1014	0.11	40	15	846	Holden (1962)	
Sweden						12	770	Stenberg (2005)	
NW Atlantic (U.S.A.)		250 **	1008	0.12	24	9	769	Bubley (2012)	from vertebra
NW Atlantic (U.S.A.)		250 **	1072	0.08	28	9	769		from spines
NW Atlantic (U.S.A.)		249	1005	0.11		12	790	Nammack (1985)	
NW Atlantic (U.S.A.)		303.5 **	1334	0.04	31	16	809	Campana (2009)	
Southern Hemisphere									
New Zealand	-3.45		1201	0.07		10	745	Hanchet (1997)	
Macquarie Harbour, Tasmania		215	1242	0.05	28	8	555	This study	VBGM
Macquarie Harbour, Tasmania		229	1153		28	8	555	This study	MMI

Length measurements from all studies have been standardized to TL using study specific length convertion equations

* L_{50} has likely been underestimated due to the methodology used to asses maturity. Smallest reported gravid female was 570 mm in length

** Fixed value based on mean size at birth

in multiple growth increments being compressed together, making interpretation of ages from vertebrae harder in the species.

Squalus acanthias in Macquarie Harbour approach their asymptotic length relatively slowly and have a longevity of at least 28 years. Growth is sexually dimorphic, with males maturing earlier, growing to smaller sizes and being less longevous (22 years) than females. Because of these differences, males grow at a faster rate than females, maturing and approaching asymptotic length at a younger age. The longevity, asymptotic length and growth rate of this population fall within the range of values reported for other populations (Table 4.8) and are like those seen in New Zealand (Hanchet, 1997) and in the northwest Atlantic (Slauson et al., 1983, Nammack et al., 1985, Campana et al., 2009). Populations in the Black Sea have been reported to reach larger asymptotic lengths and grow at higher rates (Avsar, 2001, Demirhan et al., 2007), however, these results have been questioned given that a separate study has reported values closer to those in the north Atlantic populations (Campana et al., 2009). Despite the similarities between *S. acanthias* in Macquarie Harbour and other populations, they appear to mature at

smaller sizes (Chapter 3) and ages than elsewhere. This difference is one of several aspects of the life history (i.e. reproduction, Chapter 3) and ecology (i.e spatial ecology, Chapter 2) of the Macquarie Harbour population which appear to be distinct from other populations of the species.

Differences in the reported growth characteristics of *S. acanthias*, sometimes even in the same population, suggest that some of the variability may be the result of incosistensies in age determination and possibly sampling between studies (Taylor et al., 2013). Despite this, it is likely that most of the reported variability in life-history parameters is reflective of real differences between populations. Chondrichthyan growth is a dynamic process, determined not only phenotypically (i.e. endogenous factors), but also by environmental factors (e.g. temperature, diet, prey availability) and demographic processes (i.e. density dependent responses to exploitation) (Camhi, 1998). For example, in the North Pacific, resampling of a population of *S. suckleyi* after 60 years of fishing and environmental change revealed that age at 50% maturity and asymptotic length decreased from 43 to 31 years and from 1756 to 1091 mm respectively (Taylor and Gallucci, 2009). Since fishing pressure is likely to be negligible in Macquarie Harbour (Bell and Lyle, 2016), the differences in the life-history characteristics in this population (e.g. younger maturity) may be a result of genetic expression and/or environmental influences. Indirect pressure could arise from habitat and niche constraints through the combination of environmental degradation (low trophic diversity and hypoxic conditions) (MHDOWG 2014) and anthropogenic effects (environmental degradation and dependence on provisioning from aquaculture activity) (Carpenter 1991, Ross et al., 2017).

One factor that is likely to affect the growth of the species in the area is their feeding ecology, as the current diet of *S. acanthias* in Macquarie Harbour is largely composed of spill-over feed pellets from salmonid aquaculture (Chapter 3). Aquaculture feeds are designed to maximize the growth of culture species (Mente et al., 2006, Arechavala-Lopez et al., 2015) but the effects that

such a diet may have in long-lived species like *S. acanthias* is poorly understood. Feed pellets have a high-caloric content that is likely unmatched by the prey found in the naturally depauperate harbour. Subsidies to growth provided by a pellet dominated diet could influence maturation and may explain the higher initial growth rates seen in this population, particularly early in life when energetic investment in growth is highest.

Sexual dimorphism results from sex-specific evolutionary and physiological mechanisms and has been well documented in elasmobranchs including *S. acanthias* (Orlov et al., 2011 and references therein). Although it is common for females of the species to be larger and mature at larger size/age than males, the difference in growth rates (e.g. dL/dt_{mat50}) seen in Macquarie Harbour higher than in most other populations (Bubley et al., 2012 and references therein). A similar divergence in the growth rate of male and female *S. acanthias* was reported in Ireland (Henderson et al., 2002). The authors of the study hypothesized the pattern could be the result of the removal of larger males through selective fishing (Henderson et al., 2002); however, Campana et al. (2009) suggested that this was most likely a sampling artefact, as large males occupy a different spatial range, biasing the sample towards younger individuals and misrepresenting the growth parameters. Neither of these explanations are likely to be applicable to Macquarie Harbour, as the population is not targeted by any fishery in a significant way (Lyle et al., 2014), there is no evidence of larger individuals having a distinct spatial range (Chapter 2), and the older age classes were well represented in the sample.

Most ageing studies do not consider possible underlying biases in the data set caused by age/size spatial partitioning and gear selectivity (Thorson and Simpfendorfer, 2009). Intuitively, these factors would provide the likeliest explanation for the lack of individuals <525 mm in samples from Macquarie Harbour. The types of gear used to capture fish in this study (i.e. demersal long lines and gillnets) can be highly size selective (Thorson and Simpfendorfer, 2009), which could account for the paucity of smaller individuals. However, the same gear was used to target *S.*

acanthias in Frederick Henry Bay, Tasmania, where 23 of 41 individuals caught were <525 mm (Chapter 3). Therefore, while a bias in size composition of the sample due to gear selectivity cannot be discounted, it is unlikely that this alone could explain the paucity of small size classes observed in Macquarie Harbour.

It is unclear if juvenile *S. acanthias* are resident within the estuary as the adults appear to be (Chapter 2). Spatial partitioning cannot be discounted and could explain the paucity of juveniles in the sample. However, it should be noted that despite their low incidence, individuals <525 mm were captured in this study (n=15) and past surveys (Lyle et al., 2014); providing evidence that individuals in these size classes can be, at least sometimes, found in Macquarie Harbour. Given that the harbour is shallow (>50 m) and has a strong halocline that habitually reaches depths of over 10 m, it is unlikely that segregation along the water column occurs in this population (a phenomenon reported in populations inhabiting areas with depths >200m (Shepherd et al., 2002)). As fishing effort was reasonably distributed across the study site and depths, it is unlikely that any portion of the population that was present inside the estuary would have been outside the areas sampled. Therefore, if juveniles are found to be resident in Macquarie Harbour, it is feasible that the size distribution of *S. acanthias* observed in this study is reflective of an under-representation of the younger age classes in the population. Considering that evidence suggests that the reproductive output of the population may currently be severely compromised (Chapter 3), the absence of younger individuals may imply that this issue began ~ 5 years ago.

4.4.1. Model selection and performance

No single growth model consistently outperformed the others and the w_i selection criteria did not support a single model solution in any of the data-set/sex permutations. This is suggestive of elevated model performance uncertainty and is likely caused by the gaps in the dataset (i.e. absence of the younger age classes). The MMI approach treats w_i as a Bayesian probability,

providing an estimate of the likelihood that each model represents the 'true' value. Using this value to calculate a weighted average of the growth parameters negates the need to choose a single candidate model and provides a good characterization of modelling uncertainty. In the absence of a clear superior candidate model, the model-averaged parameters provide a good compromise by incorporating uncertainty and in data poor situations as encountered in this study, should be selected over the single model estimates when informing demographic analysis or management.

Back calculated lengths can reduce uncertainty around underrepresented size classes. However, the technique is not designed to fully substitute a large portion of the size range, which could introduce additional error through pseudo-replication and variability in vertebrae growth. Given that a few females in the underrepresented size classes were present in this study, it may be preferable to use the parameters derived only from the observed data until absolute age validation can confirm the accuracy of the back calculated estimates. Conversely, for males, individuals between the ages 0 and 5 were completely absent from the sample and because of this, the models are unlikely to accurately represent the inflection in the growth curve. Therefore, back calculated estimates should be used as a tentative value in the absence of a better estimate.

Interestingly, the two-parameter (fixed L_0) VBGM was the best ranked candidate model for female growth under data-sparse conditions (observed data). The use of this parametrisation of the VBGM is attractive as it releases an additional degree of freedom, usually improving model fit. Growth parameters obtained using the two-parameter VBGM have been reported for *S. acanthias* in coastal waters of eastern Canada (Campana et al., 2009) and U.S.A. (Bubley et al., 2012). In these studies,statistical fit was used to justify the selection of the two-parameter VBGM over other candidate models. However, work comparing the two parameter VBGM with the traditional parametrization (3 parameters) has shown that although fixing the value of L_0

improves statistical fit, it may also decrease accuracy (Pardo et al., 2013). This can produce an overestimation of L_∞ and k (Thorson and Simpfendorfer, 2009), resulting in an unrealistic representation of age at length despite improved model parsimony. Furthermore, the 2-parameter model is highly sensitive to the value of L_0 used, and in data poor conditions, the assumptions that size at birth is known and that the size/age range of the data is reasonably well spread are usually unmet (Fabens, 1965), For these reasons, using a fixed L_0 VBGM to estimate the growth coefficients of *S. acanthias* should be avoided in favour of alternative models if the data permits it, even when ranked above other candidate models.

Araya and Cubillos (2006) reassessed the growth of several elasmobranch species by fitting the hyper-K model (Soriano et al., 1992) to published growth data. The biphasic model performed better than the standard VBGM in most cases, one exception being *S. acanthias* from the Black Sea. In contrast, Tribuzio et al. (2010) reported that in the sister species *S. suckleyi* (as *S. acanthias*) the two-phase model performed better than the single-phase options. In the present study, the variable performance of the two-phase models may be partly caused by the heavier penalty imposed by AIC_C on the additional parameters model (Minte-Vera et al., 2016). Interestingly, when the biphasic models were ranked favourably, phase transition was negligible, and the model approximated the single-phase VBGM. This corresponds with results from Araya and Cubillos (2006) and may suggest that unlike other elasmobranchs, the growth of *S. acanthias* does not follow a biphasic mode. However, it is possible that the sample sizes used in this study were too small for the adequate determination of the inflection point. Thus, this evaluation of these models was inconclusive, but future work in *S. acanthias*, and other species, should include cost of reproduction models, as they have the possibility of capturing additional features of growth related to life-history events.

4.4.2. Conclusion and recommendations

Vertebrae are not susceptible to wear or breakage and appear to be a superior structure for ageing. However, this technique has not been as rigorously evaluated as the use of spines, and future studies should focus on validating their applicability. Ultimately more accurate age estimates will improve age and growth models and generate more realistic life-history estimates for use in stock assessment

Although the population of *S. acanthias* in Macquarie Harbour is as long-lived as their northern hemisphere conspecifics, maximum sizes are smaller, and maturity is attained at a younger age. Recent observations (Chapter 2, Chapter 3) suggest that the population may be experiencing the effects of some level of resource limitation, or environmental perturbation, which appears to have influenced key population processes. It is possible that this has compromised the reproductive cycle resulting in a low net reproductive output (Chapter 3) and this could explain in part at least the under-representation of juvenile age classes seen in this study. The lack of historical data precludes the establishment of a historical baseline; therefore it is unknown if the traits reported herein are a long-term characteristic of the population. Nevertheless, the differences in growth between Macquarie Harbour and other populations are likely to have important consequences for the demographic dynamics of the population. In particular, maturing at younger ages could increase the reproductive life of the population, which may help them cope with some of the external pressures of environmental perturbations and density dependent processes.

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Chapter Five: General Discussion: Demographic and Risk Analysis of *S. acanthias* In Macquarie Harbour

This final chapter integrates the findings of previous chapters into a quantitative model of the population dynamics of *S. acanthias* in Macquarie Harbour. Firstly, the main findings of previous chapters are recounted, followed by a brief introduction to demographic analysis techniques and a description of the modelling framework implemented herein. In the main section of the chapter the implications of these findings for the ecology, population dynamics and management of *S. acanthias* in Macquarie Harbour are discussed, using the contextual frame of the demographic results. This is presented in two parts: firstly, the current demographic characteristics of the population are outlined and compared with other populations and species of elasmobranch; and secondly, inferences on the status of the population are presented and used in a prospective risk assessment of the ecological and management implications of changing environmental conditions in Macquarie Harbour. Finally, future research priorities are identified, and the contributions and importance of this work are summarised.

5.1. INTRODUCTION

There is growing evidence of worldwide declines in many elasmobranch populations due to exploitation, habitat loss and environmental degradation (Barausse et al., 2014, Ferretti et al., 2010, Myers et al., 2007). In response there has been an increase in research into the group and improved management of many species globally (Dulvy et al., 2008). However, elasmobranch fisheries typically have low commercial value, and population changes in biomass and mortality have not been well documented (Ferretti et al., 2010), precluding the applicability of traditional fisheries stock assessment and management tools that require such information. To overcome these limitations, the relationships between life-history parameters have been increasingly studied and used to construct demographic models that can aid management (Cortés, 2004, Frisk et al., 2005, Gedamke et al., 2007).

Due to their high abundance, broad range and historical importance to commercial fishing (Fordham et al., 2006), *S. acanthias* has been studied for a longer time, and in greater detail, than most elasmobranchs (Beamish et al., 2009). They are small demersal sharks, but their conservative life history more closely resembles that of larger pelagic species (Cortés, 2002). They are often used in comparative life-history studies between elasmobranchs as they are considered a model species for k-selected strategists (Cortés, 2002, Cortés, 2004, Frisk et al., 2001, Smith et al., 1998, Mollet and Cailliet, 2002). Although there are generalities in key life-history traits, individual demographic traits are variable. For example, while all previously-studied populations exhibit a synchronous reproductive cycle with clear seasonality, the timing of parturition and mating is different between populations (Demirhan and Seyhan, 2006 and references therein).

Perhaps paradoxically, despite the wealth of knowledge about the species, there is a general lack of understanding about their ecological role, and they continue to be a low management priority in many places (Fordham et al., 2006). This is surprising as the species is commonly understood

to be particularly vulnerable to depletion, slow to recover, and the intraspecific variability in life-history traits precludes the use of a generalized management strategy (Beamish et al., 2009). Most studies of *S. acanthias* have been on populations in the northern hemisphere and there is a lack of information on the species in its southern distribution (Gaitán-Espitia et al., 2017). In Australia, the biology and dynamics of populations of the species remain poorly understood. Although some state restrictions are afforded to bycatch and elasmobranch species, no specific management strategy is in place for *S. acanthias* in Commonwealth managed fisheries. Accordingly, this thesis focused on a previously unstudied Tasmanian population of *S. acanthias*.

5.1.1. Synthesis of main findings

The primary aim of this thesis was to provide a comprehensive assessment of the life-history characteristics of *S. acanthias* in Macquarie Harbour, Tasmania, to support and inform management. At the onset of this study, no major departures from the general biology and movement dynamics of the species were anticipated. However, our findings suggest a unique population with evidence of physiological and behavioural characteristics that deviate from the known biology of the species in important and potentially impactful ways.

Overall, in Macquarie Harbour *S. acanthias* exhibit faster early growth, mature at smaller sizes and younger ages and attain smaller maximum sizes than conspecifics elsewhere (Chapters 3 and 4). Unexpectedly, their spatial distribution is indicative of long-term residency within the system, with no evidence of emigration out of the estuary for adults of either sex (Chapter 2). Spatial dynamics of the population did not change significantly based on season, sex or life-history stage (e.g. reproductive stage). However, movement was not a random process, as evidenced by a clear affinity towards specific areas of the harbour (Chapter 2). The spatial range of males and females consistently overlapped, but behavioural avoidance between sexes

appeared to partially substitute spatial partitioning, possibly restricting the detrimental consequences of increased density (e.g. competition, cannibalism).

There was evidence for mating activity and new pregnancies developing throughout the year but not seasonally as seen in other populations (Demirhan and Seyhan, 2006). Furthermore, 70% of mature females were not in an active stage of pregnancy, and in the 30% that were, with few exceptions only the early stages of pregnancy were observed. The absence of mid or late stage pregnancies and pre-reproductive individuals, as well as the apparent long-term residency of adults inside the harbour, suggest that the populations productivity and reproductive cycle may be compromised, resulting in the premature interruption of most pregnancies. Diet was also seasonally invariant and was overwhelmingly dependent on aquaculture provisioned resources such as spillover feed pellets, escapee salmonids and sessile organisms likely detached from aquaculture cages during cleaning of the aquaculture cages (Chapter 3).

In general *S. acanthias* are highly mobile, but due to the physical (i.e. narrow and shallow entrance) and environmental characteristics (i.e. stratification of the water column) of Macquarie Harbour (Cresswell et al., 1989) movement may be effectively restricted. Range restriction could compromise the adaptive ability of this population, making them more susceptible to environmental perturbations and habitat degradation. It is likely that the unique life-history traits of *S. acanthias* have complex mechanistic links to the environmental conditions and anthropogenic stressors in Macquarie Harbour. Two potentially important risk sources were identified, 1) widespread reproductive failure, and 2) a dietary dependence on aquaculture operations. The species is also subject to limited fishing pressure, although in its current form the fishery is unlikely to represent a source of risk. Thus, the formulation of predictive tools that can provide insight into the demographic dynamics of this population is of great importance. In the following sections, a demographic model of the Macquarie Harbour population is constructed using available life-history information, thereby providing the tools

necessary to facilitate prospective and retrospective analysis of the status of this unique population and to help identify priority areas for future research.

5.2. MODELLING FRAMEWORK

Knowledge of population dynamics is particularly informative for fisheries management as it provides insight into temporal patterns of population abundance, while recognizing the individual variability of biological and ecological process that affect a species through its ontogeny (Cortés, 2004). Traditional quantitative stock assessment frameworks are used to manage both teleost and elasmobranch fisheries; however, they require a large amount of data, which is rarely available for non-target elasmobranch species (Simpfendorfer, 2005). Further, elasmobranchs and other species with conservative life histories can violate some of the core assumptions in these models, most importantly, that changes in stock density have an immediate effect on the growth rate of the population (r) (Gedamke et al., 2007).

Life-history traits are linked to population dynamics and these relationships can be used to perform a demographic analysis (Cortés, 2002). This approach only requires knowledge of basic life-history parameters, making it a valuable tool for data-limited species (Brooks et al., 2009, Simpfendorfer et al., 2008). Otherwise known as cohort-structured models, they can be constructed around stage or age, and model the instantaneous growth rate (r) of a population (Simpfendorfer, 2005). Here, an age-structured Leslie Matrix formulation (Caswell, 2001) was chosen as the underlying framework for the model. Although the basic implementation of this popular approach is deterministic, uncertainty can be incorporated in vital rates using Monte Carlo simulation (Cortés, 2002). The treatment of demographic rates as stochastic processes recognizes that these traits are unlikely to be static as well as acknowledging the difficulty and error associated with measuring them. This probabilistic approach can also be used to generate a prior distribution of r to be used in Bayesian stock assessment (Cortés, 2004).

Demographic modelling is only useful when the results produce biologically meaningful information that can be used in management (e.g. biomass, recovery times, intrinsic growth rates). As such, in addition to the standard metrics commonly presented in demographic studies

Table 5.1. Models used to estimate natural mortality indirectly from life-history parameters. Age specific values used for each iteration of the Monte Carlo simulation were calculated randomly using a triangular distribution.

Source	Function	Comments
Age-independent		
Hisano (2011)	$M = \frac{1.65}{t_{mat} - t_0}$	Modified to allow for size at birth > 0 from Jensen (1996)
Jensen (1996)	$M = 1.5k$	
Frisk (2001)	$\ln(M) = 1.1\ln(k) - 0.8$	
Chen & Yuan (2006)	$\ln(M) = 1.46 - 1.01 * \ln(t_{max}),$ $t_{max} = t_0 - \frac{\ln(0.05)}{k}$	Assuming that $t_{max} \approx 0.95L_\infty$
Pauly (1980)	$\log(M) = -0.0066 - (0.279 * \ln(L_\infty)) + (0.6743 * \ln(k)) + (0.4634 * \log(Temp))$	Mean temperature from Bell et al. (2016)
Gunderson & Dygert (1988)	$M = 0.03 + 1.68\, Ig,$ $Ig = ovarymass(W)^{-1}$	Using wet weights
Age-dependent		
Peterson & Wroblewski (1984)	$M_{Wt} = 1.92\, Wt^{-0.25}$	
Chen & Watanabe (1989)	$M_t = \begin{cases} k/(1 - exp^{-k(t-t_0)}), & t < t_m \\ k/(a_0 + a_1(t - t_m) + a_2(t - t_m)^2), & t \geq t_m \end{cases}$ where $\begin{cases} a_0 = 1 - exp^{-k(t_m - t_0)} \\ a_1 = k\, exp^{-k(t_m - t_0)} \\ a_2 = -0.5k^2\, exp^{-k(t_m - t_0)} \end{cases}$ $t_m = \frac{1}{k}\ln(1 - exp^{kt_0}) + t_0$	

(i.e. intrinsic growth rate, generation time, net reproductive output), analytically derived reference points have been provided to be used for comparative purposes with other demographic assessments and stock surplus models (Brooks et al., 2009). A definition of these metrics and a detailed methodological description of model implementation and construction can be found in Appendix 3. Perhaps the biggest limitation of these models is that they assume time-invariance and do not include density dependence (Caswell, 2001). Accordingly, the results from each model represent only a snapshot of the population. Thus, a set of four scenarios (detailed below) were modelled to analyse the effects of varying input parameters, either through natural variability or hypothetical scenarios.

Table 5.2. Distribution of input parameters used in the stochastic demographic models of *S. acanthias* in Macquarie Harbour. Growth coefficients from the VBGF, age at maturity (α), max reproductive age (w), natural mortality (M) and fecundity were derived from this study. Gestation length and litter sex ratios were assumed to be consistent with values reported in the literature for the species elsewhere (Ketchen 1975)

Parameter	Unit	Distribution		sd / range
L_0	mm	multivariate normal	229	6.5
L_∞	mm		1153	66.1
k	yr^{-1}		0.08	0.007
α	yr	normal	8.54	0.23
w	yr	uniform		28-t_{max}
M		triangular		
f		triangular	8	1-13
gestation	yr		2	
sex ratio			0.5	

Table 5.3 Summary of modelled conditions for fishing mortality (F), fecundity (f) and resting period between subsequent pregnancies in each of the 4 scenarios. Age of entry into the fishery or age at first capture (AAFC). Na= not applicable

	Fishing mortality		Fecundity		Rest period	
Scenario	F	AAFC	Mode	Range	Length	Probability
1 Baseline	0	Na	8	(1-13)	Na	Na
2 Fishing	0-1	0-t_{max}	8	(1-13)	Na	Na
3 Sensitivity	0	Na	8	(1-13)	Na	Na
4 Reduced fecundity	0	Na	2.4	(1-3.9)	1 yr	50%

5.2.1. Scenario 1 - Baseline

This age-structured model was based on the current demographic characteristics of *S. acanthias* in Macquarie Harbour as reported in this thesis (Chapter 3 and Chapter 4). The probability distributions used to characterize uncertainty around estimated age and growth parameters were constructed using the error terms from multi model inference (Chapter 4). Natural mortality was derived from life-history data using eight different models (Table 5.1), resulting in an age specific statistical distribution. Since fishing mortality (F) is unknown, in this scenario it was assumed that F=0, and total mortality for the 'virgin population' (Z) was assumed to equal natural mortality (M), which was estimated indirectly (as per Simpfendorfer, 1999). Gestation length could not be directly determined, so a 2-year gestation was assumed based on published estimates for the species.

5.2.2. Scenario 2 – Fishing effects and risk assessment

This scenario aims to predict the impacts that future perturbations or management strategies could have on the population dynamics of *S. acanthias* and determine the theoretic limits of the population's resilience to harvesting or any other activity that increases mortality (McAuley et al., 2007). Impacts were modelled as an increase in mortality rates (F) through an iterative analysis to determine the constant harvest rate necessary for the population growth rate to reach zero (critical fishing mortality or F_{crit}). Size-selective impacts were modelled through the introduction of an age at first capture (i.e. age at which the individual first becomes susceptible to an impact or enters the fishery).

5.2.3. Scenario 3 – Elasticity and sensitivity analyses

Isolating the relative impact that stochasticity and input variability will have on the modelled demographic parameters of the population is useful for identifying management and research priorities (Heppell et al., 2000). Elasticities are a property of the projection matrix (i.e. right eigenvector of the transpose matrix) and represent a dimensionless proportional measurement of the model's sensitivity (Carslake et al., 2009). Essentially, elasticities show the proportional change in r that would result from a small perturbation on the survivorship of each life stage. Similarly, partial correlation analysis identifies which of the input parameters accounts for the most variability in r. This is an indication of how robust the model is to uncertainty and can be used to identify priority areas for future research that would increase the accuracy of the model.

5.2.4. Scenario 4 – Reproductively compromised population

Gestation patterns seen in Macquarie Harbour may be indicative of reproductive failure for female *S. acanthias* (Chapter 3) resulting in an acutely compromised reproductive output, which will affect the growth potential of the population. Although further research is needed to corroborate this, and the potential magnitude of this effect is unknown, if pregnancies are discontinued prior to parturition, fecundity estimates based on uterine egg counts are not an

adequate reflection of productivity. To simulate the hypothetical effects of widespread reproductive failure, a 70% decrease in population fecundity (0.3 f) was chosen based on the observed distribution of pregnancy stages in the population (Chapter 3). Likewise, a 50% probability of females skipping a year between subsequent pregnancies was introduced to simulate a resting stage (Chapter 3).

The output coefficient of demographic models is commonly reported as the intrinsic growth rate of the population (i.e. growth rate in the absence of fishing and at low population sizes) (Cortés, 2004, Gedamke et al., 2007). For *S. acanthias* in Macquarie Harbour it is unknown if these conditions are met, and thus it may be more appropriate to consider our results as the short-term growth rate of the population given the present conditions, rconditional instead of rintrinsic. The value of r is related to population size and the conditions at which the demographic parameters of the population were estimated (i.e. stock exploitation level and depletion from virgin conditions) (Gedamke et al., 2007). Thus, in the future these results can be combined with repeated observations of the same population to analytically derive rintrinsic and K (carrying capacity).

5.3. POPULATION DYNAMICS OF *S. ACANTHIAS* IN MACQUARIE HARBOUR.

Results from the baseline model suggest that *S. acanthias* in Macquarie Harbour do not fit the 'extremely unproductive' descriptor conventionally associated with the species in the literature (Cortés, 2002, Smith et al., 1998, Smith et al., 2008). The population's instantaneous growth rate (r) results in positive population growth despite modelling uncertainty (r=0.09, c.i.95=0.02-0.15), and it appears to be able to sustain moderate harvest rates before r reaches 0 (F_{crit}=0.2) (Scenario 2). Although higher than in other *S. acanthias* populations, the productivity of this population is still low when compared to other small coastal shark species such as the Australian sharpnose shark (*R. taylori*) (Simpfendorfer, 1999). The baseline model of *S. acanthias* in Macquarie Harbour suggests that the maximum production net change for the population occurs at 57% of K (i.e. carrying capacity) (Figure 5.1). This has two important implications: first, that compensatory responses in the population will be delayed (i.e. delayed density compensation) and therefore recovery times will be longer; and second, the magnitude

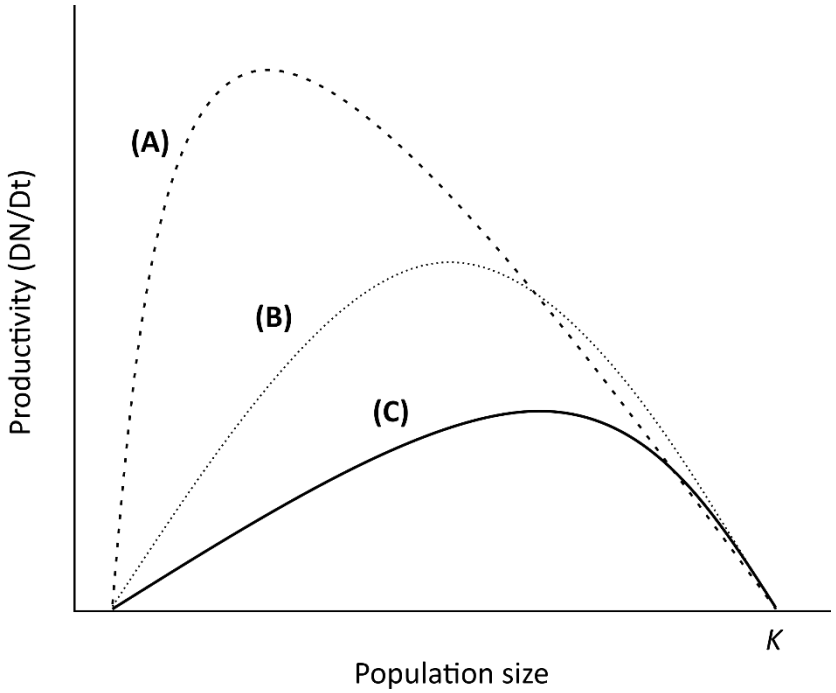


Figure 5.1. Change in the productivity of a population in response to population size towards carrying capacity (i.e. K) for an r-strategist species (**A**), normal logistic growth (**B**) and *S. acanthias* in this study, a k-strategist (**C**).

of the response capacity of these sharks will be smaller than for more productive species (Cortés, 2004).

A focal challenge for the present study is the absence of historical data on abundance, demographics or life history. This framework is retrospective because it evaluates the current demographic characteristics of the population, which have been dynamically shaped by past conditions. However, the absence of past data means that elucidating the causal attribution that environmental and anthropogenic conditions have had on those characteristics is nearly impossible (Taylor and Gallucci, 2009). Furthermore, while the effects of past perturbations are largely incorporated into the analysis through their effect on the life-history parameters, recent or ongoing perturbations will not be incorporated. Thus, this scenario can be interpreted as the current demographic potential for the population under ideal conditions (i.e. assuming stable age, and no further perturbations).

5.3.1. Comparison with other populations and species

Non-traditional reference points, like intrinsic rebound potential, can be used to directly compare the productivity of different species or populations (Simpfendorfer, 2005). Based on fisheries dynamics theory, this method uses a simplified model to simulate r at maximum sustainable yield (MSY) (assumed to occur at 1.5M or 2M (Au et al., 2015)), when the species productivity is maximized (Au et al., 2015). Density dependence is incorporated, although it is assumed to occur exclusively as a compensatory increase in pre-reproductive survivorship (Smith et al., 1998). In Macquarie Harbour, *S. acanthias* showed a growth rate at MSY (r2M) of 0.042, assuming a survival to maturity ($l_{a,2M}$) of 0.138. Comparing these results with published multi-species reviews reveals that *S. acanthias* in Macquarie Harbour are more productive than other populations of the same species, for example, their intrinsic rebound potential was 27% higher than *S. acanthias* in the north Atlantic (r2M=0.034) and 152% higher than *S. suckleyi* (as *S. acanthias*) in the north Pacific (r2M=0.017) (Au et al., 2015, Smith et al., 1998, Smith et al.,

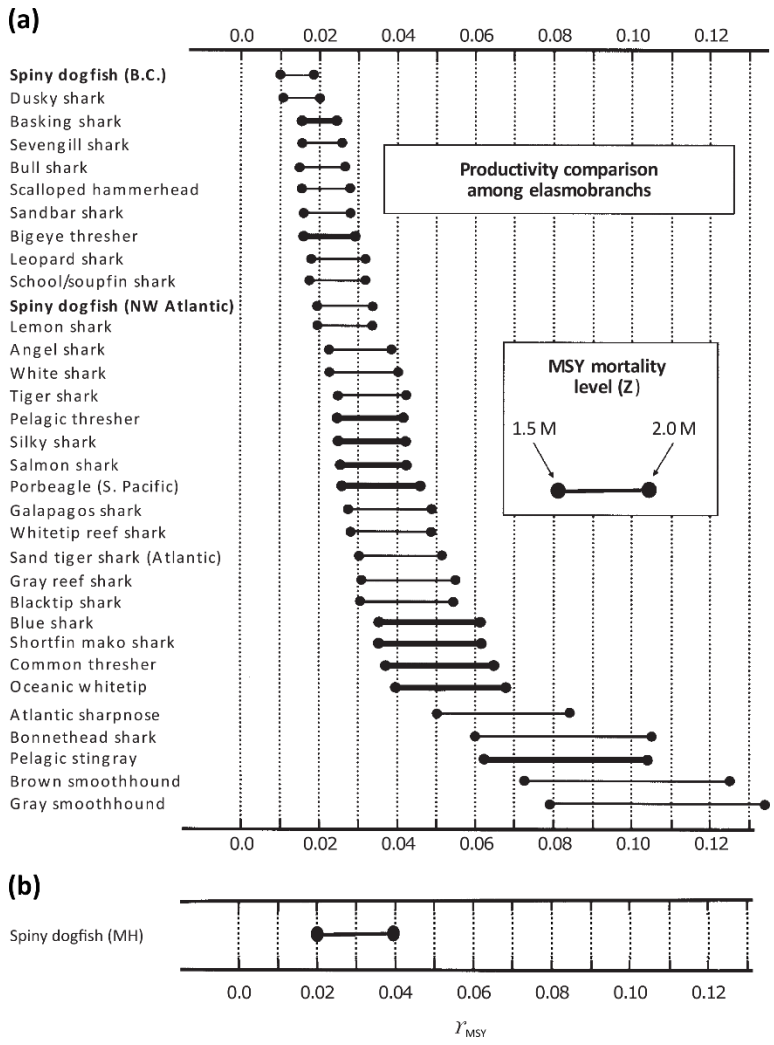


Figure 5.2. (a) Intrinsic rebound potential for multiple elasmobranch species. Figure reproduced from Camhi et al. (2009) with data from non-pelagic species from Smith et al. (1998) and Au et al. (2008). **(b)** Intrinsic rebound potential of *S. acanthias* in Macquarie Harbour (this study)

2008). Instead, the rebound potential of this population more closely resembled that of large pelagic species like salmon (*Lamma ditropis*) and silky sharks (*Carcharhinus falciformis*), which occupy the middle range of the r-continuum (Figure 5.2). In contrast, results from Scenario 4 indicate that despite having a higher rebound potential than their conspecifics, the effects of widespread compromised reproductive output may overwhelm the recovery capacity of the population (see reproductive biology section below).

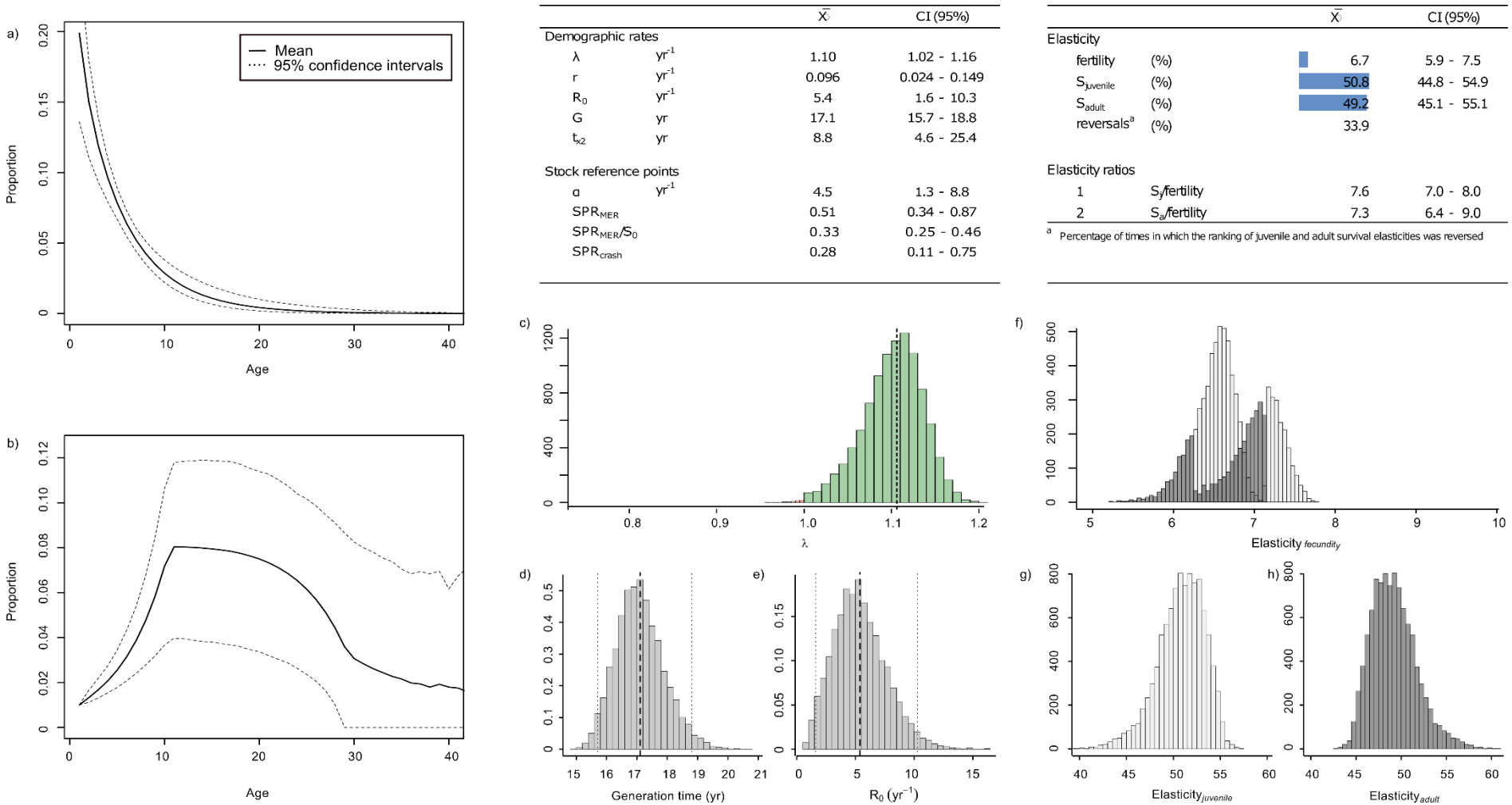
In 2010, *S. acanthias* populations in the North Pacific were identified as a separate species and renamed as *S. suckleyi* (Ebert et al., 2010). In studies published prior to the separation of the species, there are generalizations and traits that are commonly associated with *S. acanthias* but

pertain to *S. suckleyi* instead. For example, values derived in the North Pacific are often used to exemplify the very conservative life history strategy of the species, as they mature as late as 35 years and live past 80 years (Ketchen, 1975). However, despite being morphologically similar, the life-history characteristics of *S. acanthias* are not as extreme, maturing between 8 and 15 years and capable of a maximum empirical age of 40 years (Orlov et al., 2011, Taylor and Gallucci, 2009, Wood et al., 1979). Because of these differences, the species should be assessed separately, and current strategies revised. For example, although the IUCN has recognized the separation of the species, the latest red list assessment of *S. acanthias* was still informed by demographic parameters (i.e. generation time) derived from datasets that included both species (Fordham et al., 2006).

5.3.2. Risk assessment and prospective perturbation analysis

Elasticity analysis showed that changes in juvenile survivorship had, on average, the greatest impact on r, and fecundity had the smallest (Figure 5.3). This is because slow growing species that mature at older ages have a lower probability of survival to reproductive age but tend to have a longer lasting reproductive period (Cortés, 2004). This trade-off helps bridge years when recruitment or survival may be especially compromised due to environmental variability (Frisk et al., 2005). By maturing earlier, a larger portion of the population of *S. acanthias* in Macquarie Harbour is likely to survive to a reproductive age, increasing the productivity of the population but making the shortened juvenile stage less important (Figure 5.3). In 34% of simulations, the ranking between juvenile and adult elasticity was reversed in favour of adult survivorship. To compensate for a 10% removal of juveniles or adults, fecundity in the population would have to increase by approximately 75% (Figure 5.3); this compares with *S. acanthias* of the northwest Atlantic, where an increase of 82% and 115% would be required to compensate for an equivalent removal of juveniles or adults respectively (Cortés, 2002). Moreover, fecundity of the species has been shown to increase with maternal size (Demirhan and Seyhan, 2006); thus, although maturing at a younger age will allow for a longer period of reproductive activity, the smaller

maximum size of *S. acanthias* in Macquarie Harbour will likely decrease the fecundity of older individuals thereby limiting their reproductive contribution. Overall these results suggest that



		$\bar{X}$	CI (95%)
Demographic rates			
λ	yr^{-1}	1.10	1.02 - 1.16
r	yr^{-1}	0.096	0.024 - 0.149
R_0	yr^{-1}	5.4	1.6 - 10.3
G	yr	17.1	15.7 - 18.8
t_{x2}	yr	8.8	4.6 - 25.4
Stock reference points			
α	yr^{-1}	4.5	1.3 - 8.8
SPR_{MER}		0.51	0.34 - 0.87
SPR_{MER}/S_0		0.33	0.25 - 0.46
SPR_{crash}		0.28	0.11 - 0.75

		$\bar{X}$	CI (95%)
Elasticity			
fertility	(%)	6.7	5.9 - 7.5
$S_{juvenile}$	(%)	50.8	44.8 - 54.9
S_{adult}	(%)	49.2	45.1 - 55.1
reversals[a]	(%)	33.9	
Elasticity ratios			
1	S_j/fertility	7.6	7.0 - 8.0
2	S_a/fertility	7.3	6.4 - 9.0

[a] Percentage of times in which the ranking of juvenile and adult survival elasticities was reversed

Figure 5.3. Demographic assessment of *S. acanthias* in Macquarie Harbour using Monte Carlo simulation (Scenario 1). (a) Theoretical stable age distribution (right eigenvector). (b) Reproductive contribution per age under stable age conditions (left eigen-vector). (c, d, e) Population instant growth rate λ (leading eigenvalue), generation time (G) and net reproductive value R_0. (f, g, h) Matrix elasticity values for fecundity, juvenile survivorship and adult survivorship. The tables represent the summary statistics for all the demographic rates calculated. SPR reference points calculated analytically from the life-history data at max excess recruitment (MER), biomass depletion at MER (SPR_{MER}/S_0) and SPR as the level of spawners approaches the origin (crash).

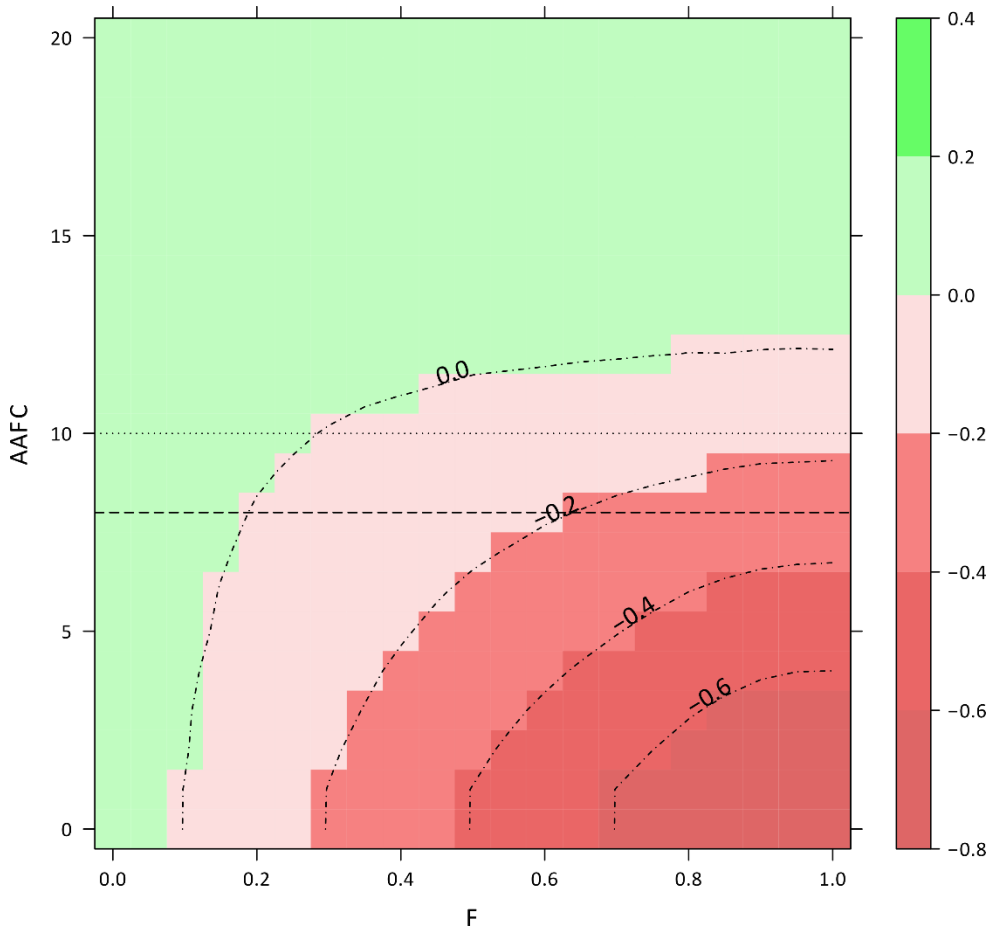


Figure 5.4. Population growth at increasing levels of harvest (F) and variable age of entry into the fishery (AAFC). Contour lines represent F at r=0 (F_{crit}) and at subsequent decline rates (20% increments). --- age at 50% maturity, ... age at first reproduction (t_{50} + gestation)

in Macquarie Harbour, *S. acanthias* will: 1) be more impacted by changes in fecundity than other populations; and 2) future compensatory responses through increases in juvenile survivorship or fecundity may be beyond the biological limits of the species (Figure 5.3).

Modelled productivity was dramatically impacted by increased mortality when pre-reproductive individuals were affected. Delaying the entry age into the fishery simulated size selective increased mortality and had a strong effect on the sustainable level of fishing pressure that the population was able to cope with (Figure 5.4). Simulating mean knife edge selection to the fishery, based on age/size at first reproduction (t50 + gestation = 10 yrs.) resulted in an exponential increase in the fishing pressure tolerable by the population (Figure 5.4). Thus, a size restriction approach (i.e. min size limits based on average age at first reproduction) could be highly effective in managing this population if required in the future. However, in Macquarie

Harbour risk of increased mortality is likely to be driven by environmental pressures rather than harvesting. Thus, the limited extent of the harbour and overlapping distribution of *S. acanthias* of both sexes indicate that spatial management strategies are unlikely to be effective unless the entire harbour is protected.

5.4. IMPLICATIONS FOR ECOLOGY, CONSERVATION AND MANAGEMENT

Taken alone, results from the baseline model (Scenario 1) and risk assessment (Scenario 2) suggest that *S. acanthias* in Macquarie Harbour are more productive than their conspecifics. This implies a positive outlook for the population considering that exploitation through fishing is minimal (Lyle et al., 2014). Despite this, some of the observations reported in this study suggest that the population may be under acute stress from non-fishing factors (e.g. resource limitation, widespread reproductive failure) and that some of the unique characteristics of their environment may increase their vulnerability to risk factors (e.g. range restriction, stock isolation, environmental degradation).

5.4.1. Range restriction

Perhaps the most unexpected, but potentially consequential finding of this study was the long-term residency of *S. acanthias* in Macquarie Harbour (Chapter 2). Design of the acoustic monitoring experiment was conceived under the assumption that *S. acanthias* is a migratory species with distinct spatial partitioning between sexes. However, individuals of both sexes were found within Macquarie Harbour throughout the year and often had overlapping ranges. Moreover, no emigration outside of the estuary was detected, suggesting the population is potentially isolated, although external seeding cannot be discounted. Unequivocally proving isolation is difficult due to the long life-span of the species and was ultimately beyond the scope of this study. Therefore, it is important to recognise that other mechanisms may be responsible for the spatial dynamics seen in this study. For example, aquaculture resource provisioning has been reported to temporarily affect the migratory behaviour in highly mobile fish species in

Europe, causing a temporary shift in spatial dynamics (Abaad et al., 2016). As such, it is possible that the development of the aquaculture industry over the last decade or so has increased the degree of site fidelity displayed by *S. acanthias*. However, in Macquarie Harbour, several aspects of the life history and ecology of *S. acanthias* provide evidence that the population may have been isolated for a long period of time:

Observed residency: Acoustically tagged individuals of both sexes were all detected continuously inside Macquarie Harbour over 15 months. Listening stations deployed in Macquarie Harbour for an unrelated study, some 9 months after the completion of this study (Dec 2015), detected the presence of one male and two females that had been tagged with long-life transmitters. Likewise, despite low fishing effort, 3 recaptures (days at liberty = 1099, 357 and 90) have been reported by researchers (personal obs.) of conventionally tagged individuals caught and released in the area concurrently with acoustic tracking. Although not proof of continuous residency, these results confirm that these individuals were still present in Macquarie Harbour well beyond the completion of the present field study.

Site-specific behavioural adaptations: State-transition models indicate that the movement of the species in the harbour is non-random, suggesting that the species has the capacity for directional movement. Site affinity was highest in the central area of the harbour for both sexes during all seasons, likely indicating active site association and habitat preferences. Likewise, patterns of social association suggest that the population has developed a behavioural mechanism for avoidance between males and females in lieu of spatial partitioning (Chapter 2).

Dietary tracers: The isotopic signatures in the muscle tissue of *S. acanthias* in Macquarie Harbour were consistent with the pellet-based diet evidenced by stomach content analysis. Given the 95% turnover estimate in muscle (488 d), dietary shifts can take over a month to present (e.g. from a marine diet to a pellet dominated diet due to emigration from offshore sites) (MacNeil et al., 2005). This acts as an alternative mid-term tracker and implies that none the

individuals included in the isotope sample had fed significantly on alternative prey sources within at least a month of being sampled (Chapter 3).

Past catch reports: Research surveys conducted prior to the present study yielded similar results in terms of size and sex information, including the lack of juveniles (Lyle et al., 2014) – which if gear selectivity can be discounted and pregnant females do not pup outside of MH – would suggest the present population structure is not an entirely new phenomenon.

Resource limitation: The general paucity of natural prey in the diet of *S. acanthias* in Macquarie Harbour, and high frequency of empty stomachs suggests a degree of resource-limitation. *Squalus acanthias* is known as a generalist feeder with high mobility and has been observed to respond to resource limitation through dietary or spatial shifts (Alonso et al., 2002). The lack of evidence for either in Macquarie Harbour may be indicative of an inability to do so (i.e. geographic isolation). Noting that even in its natural state Macquarie Harbour represents a relatively depauperate habitat in terms of biodiversity (Cresswell et al., 1989), it is unclear how the population was sustained before aquaculture provisioned resources were available.

Physical barriers: The harbour is connected to the ocean through a narrow, shallow channel and due to the high freshwater inputs into the harbour throughout much of the year, this channel is above the halocline much of the time. During flushing events, when oceanic water enters the harbour, the density differential creates a strong inward flow (Chapter 1). Given that *S. acanthias* has low physiological tolerance for low salinity conditions (Shepherd et al., 2002), the geomorphology and physio-chemical characteristics of Macquarie Harbour may create an effective barrier that restricts or negates *S. acanthias* from exiting the system.

Collectively, these observations suggest that *S. acanthias* remains resident within Macquarie Harbour for protracted periods and potentially represent an isolated population. The presence of unique behavioural responses and life-history traits suggests that these are not a recent development and thus cannot be explained as a momentary shift in biological responses to the

feed provisioning provided by the introduction of finfish farming in Macquarie Harbour that commenced in the late 1980s and expanded rapidly from 2009 on (Ross and MacLeod, 2017).

5.4.2. Demographic isolation

The area of Macquarie Harbour is much smaller than spatial ranges used by *S. acanthias* populations elsewhere (Carlson et al., 2014, Hanchet, 1986, Haugen et al., 2017, Holden, 1965, Sulikowski et al., 2010, Thorburn et al., 2015), and the inhabitable area below the halocline likely further constrains the available habitat (Chapter 2). While range shifts, range expansions and spatial partitioning are common adaptive behaviours in elasmobranchs (Sims, 2003), range-restricted species are incapable of utilising these strategies. The spatial ecology of a species is determined by a long-term trade-off between optimal foraging (e.g. prey availability) and ecological advantages (e.g. reduced competition through spatial partitioning, predator protection, mate access, thermal preference) (García et al., 2008). For example, in some elasmobranch species the use of small coastal areas as nurseries offers protection to pupping adults and early-life stages at the cost of limited prey availability (Heupel et al., 2007). The high mobility potential of *S. acanthias* and their lack of ecological specialisation is likely why the species has been so successful over such a wide geographic range, allowing behavioural strategies like spatial partitioning and life stage specific migrations (Haugen et al., 2017, Thorburn et al., 2015). These characteristics confer the species high plasticity, which can aid when coping with disturbances, as evidenced by the post-exploitation rebound of some severely depleted stocks in the northern hemisphere (Fordham et al., 2006). Long-term residency in Macquarie Harbour does not immediately appear to offer any life stage specific advantages to the species. Instead, it is likely to constrict their ability for adaptive responses through variation in spatial ecology (i.e. range shifts, spatial segregation, niche partitioning), therefore increasing their vulnerability to habitat degradation and density dependent effects (e.g. lack of sexual segregation may result in increased competition, cannibalism or reproductive harassment).

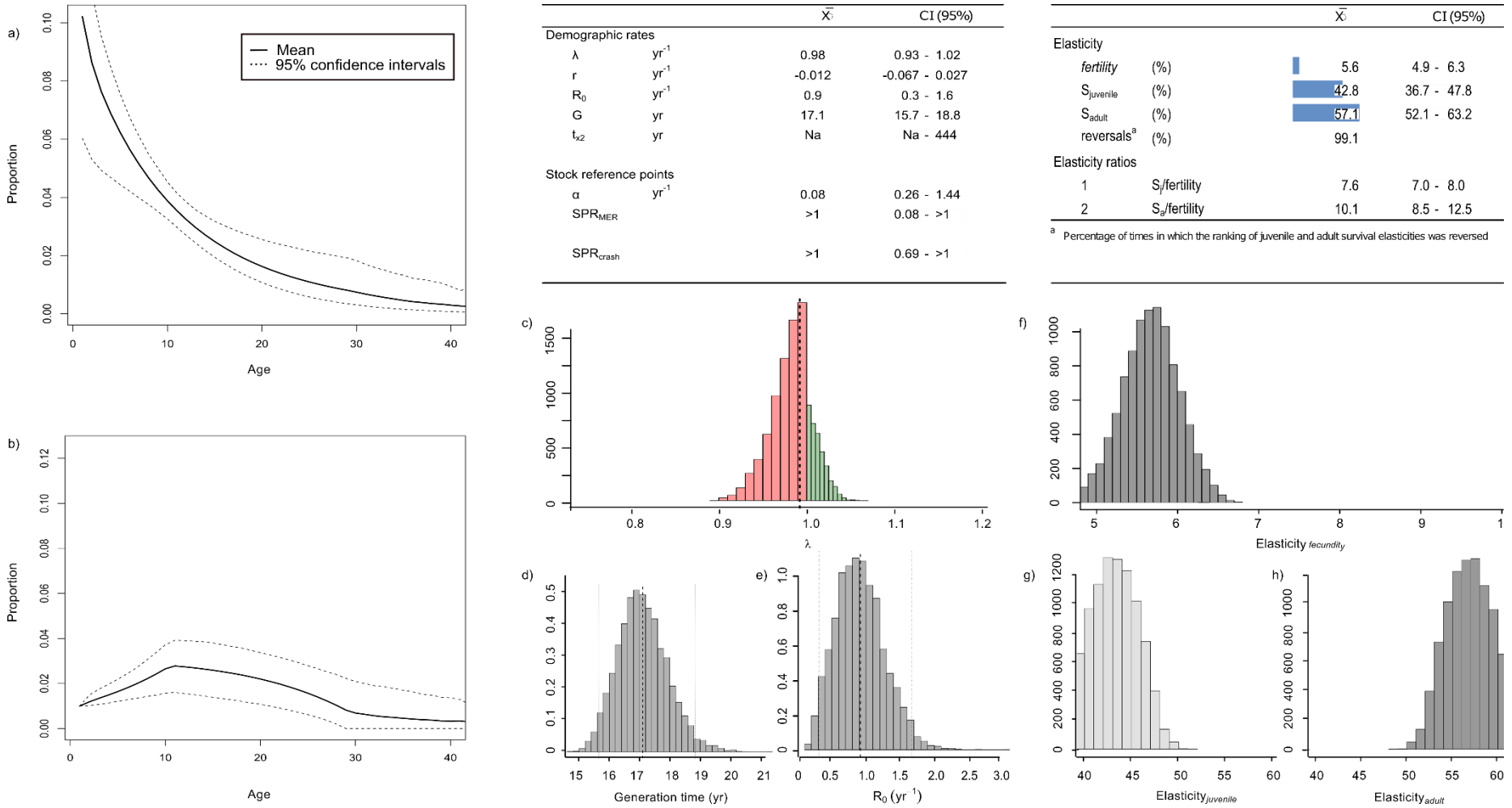
There is an important distinction to be made between an isolated management unit and an evolutionarily isolated population. For the latter, a much longer temporal scale is needed, and isolation must be nearly absolute, as 1% mixing from another population per-generation is sufficient to ensure that no genetic separation occurs (Ebert et al., 2010). Conversely, a demographically isolated unit with distinct life-history characteristics can arise even when mixing is as high as 2% annually (Tribuzio and Kruse, 2011). *Squalus acanthias* in Macquarie Harbour appear to conduct their entire reproductive cycle inside the system; however, it is possible that some amount of external seeding occurs if individuals from adjacent populations swim into the harbour. It is unclear if *S. acanthias* in Macquarie Harbour truly constitute an isolated population and if so, at what scale. However, given that the harbour appears to play a significant role in the life history of the population, and that their demographic characteristics are distinct, it is advisable that the population be treated as an isolated unit for management purposes.

5.4.3. Reproductive biology and population dynamics

Reproductive patterns of *S. acanthias* described in this study are evidence of a departure from the standard reproductive model ascribed to the species elsewhere. Females do not appear to migrate off-site in relation to their reproductive cycle (Chapter 2) and both newborns and late-stage pregnant females have been observed in the system previously (J. Bell, unpublished data). Therefore, considering the absence of individuals <6 years and that only one late stage pregnancy was observed during 1.5 years of sampling, the population is likely to be reproductively compromised (Chapter 3). If *S. acanthias* are truly geographically isolated, the effects of reproductive failure will most likely have an important impact on their population dynamics. While the extent of this problem is unknown, the values chosen in scenario 4 simulate how such a decline in productivity may affect the dynamics of the population.

Scenario 4 simulated a 70% chance for pregnancy failure and a 50% chance for a resting period of a year to occur between subsequent pregnancies. Under these conditions, despite having a long active reproductive stage, the net lifetime contribution of an average individual would not be enough to replace itself (R_0=0.9). Thus, if conditions remain unchanged, *S. acanthias* will likely experience a general reduction in population size as evidenced by negative mean growth rates (r=-0.02). However, a portion of the r distribution (~30 %) from the stochastic simulations resulted in population equilibrium or moderate growth (Figure 5.5). This indicates that the population may be able to cope with a critical reduction in recruitment for a short time; but in the long term, these conditions are unsustainable and could lead to population collapse (Figure 5.6).

Reference points based on surplus recruitment for Scenario 4 demonstrate analytically that the simulated population is consistent with an overexploited status (SPRMER/S0 > 0.5) (Brooks et al., 2009). This suggests that under reduced recruitment conditions, the population would be unable to cope with any level of exploitation or increased mortality. The ranked elasticities for juveniles and adults were reversed in ~99% of the simulations for the low reproductive output scenario (i.e. adult elasticity was higher than juvenile elasticity) (Figure 5.5). Elasticity ratios from Scenario 4 indicate that a 10% decrease in adult survivorship would require a compensatory increase in fecundity ranging from 80-120%. Furthermore, if the interruption of pregnancies is a physiological response to external factors (i.e. environmental conditions, resource limitation or adverse effects from long term feed pellet consumption), compensatory increases in fecundity or survivorship would be ineffective. The reproductive strategy of aplacental viviparous elasmobranchs results in offspring with increased survivorship (Carrier et al., 2012); but the benefits of giving offspring energetic care is negated if pregnancies are not being carried to full term. Until further research is conducted to validate if *S. acanthias* pregnancies are failing, and to what extent, protecting neonates and pregnant females should be an immediate research and management priority in Macquarie Harbour.



		$\bar{X}$	CI (95%)
Demographic rates			
λ	yr^{-1}	0.98	0.93 - 1.02
r	yr^{-1}	-0.012	-0.067 - 0.027
R_0	yr^{-1}	0.9	0.3 - 1.6
G	yr	17.1	15.7 - 18.8
t_{x2}	yr	Na	Na - 444
Stock reference points			
α	yr^{-1}	0.08	0.26 - 1.44
SPR_{MER}		>1	0.08 - >1
SPR_{crash}		>1	0.69 - >1

		$\bar{X}$	CI (95%)
Elasticity			
fertility	(%)	5.6	4.9 - 6.3
$S_{juvenile}$	(%)	42.8	36.7 - 47.8
S_{adult}	(%)	57.1	52.1 - 63.2
reversals[a]	(%)	99.1	
Elasticity ratios			
1	S_j/fertility	7.6	7.0 - 8.0
2	S_a/fertility	10.1	8.5 - 12.5

[a] Percentage of times in which the ranking of juvenile and adult survival elasticities was reversed

Figure 5.5. Demographic assessment of *S. acanthias* under reduced productivity conditions in Macquarie Harbour using Monte Carlo simulation (Scenario 4). (a) Theoretical stable age distribution (right eigenvector). (b) Reproductive contribution per age under stable age conditions (left eigen-vector). (c, d, e) Population instant growth rate λ (leading eigenvalue), net reproductive value R0 and generation time (G). (f, g, h) Matrix elasticity values for fecundity, juvenile survivorship and adult survivorship. The tables represent the summary statistics for all the demographic rates calculated. SPR reference points calculated analytically from the life-history data at max excess recruitment (MER) and SPR as the level of spawners approaches the origin (crash).

5.4.4. Diet and resource limitation

Squalus acanthias is an opportunistic generalist feeder (Fordham et al., 2006), therefore, it was not unexpected for them to feed on spillover aquaculture feed pellets or escapee salmonids (Chapter 3). It has been suggested that in generalist feeders, stomach fullness can be a good indicator of prey availability and time between feedings (Alonso et al., 2002). Based on past dietary studies of *S. acanthias* (Alonso et al., 2002, Gaitán-Espitia et al., 2017, Tanasichuk et al., 1991, Hanchet, 1991), it was expected that a portion of individuals in Macquarie Harbour would have empty stomachs, as intermittent periods of starvation between feeding events are common in species that feed opportunistically (Wood et al., 2010). However, compared to those populations (Chapter 3), the prevalence of empty stomachs was much higher in Macquarie Harbour (68%). Results from diet analysis presented in Chapter 3 highlight the strong dependence that the species has on anthropogenically provisioned resources in the area, and

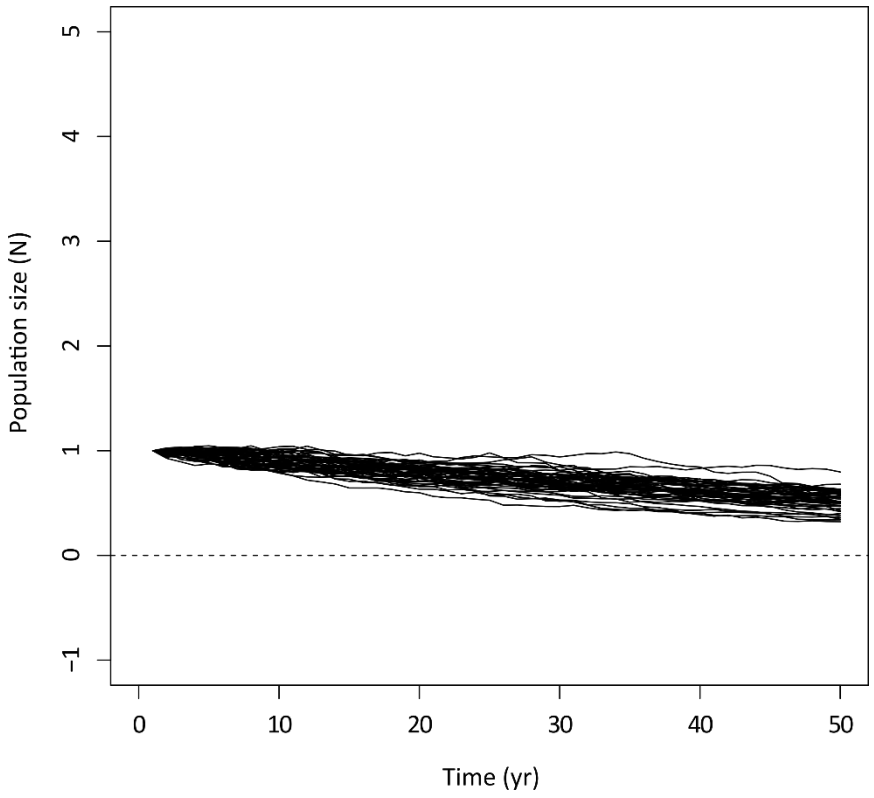


Figure 5.6. Stochastic population growth projections over a 50 yr timespan simulated the reduced fecundity model. Growth rates for 100 iterations resampled yearly from distributions calculated using Monte Carlo simulation (Scenario 4)

strongly suggest there are limited natural prey alternatives. Therefore, aquaculture provisioned resources may act as a limiting resource for the Macquarie Harbour population.

The availability of pellets in Macquarie Harbour is determined by the total production biomass held by the farms, harvesting cycles, feeding efficiency and regulatory action (DPIPWE, 2012). Under the current regulatory framework for the industry, biomass restrictions are imposed on the farms when pre-defined environmental thresholds are exceeded (Ryan, 2017). Since 2009, there has been a downward trend in dissolved oxygen levels of deep waters in Macquarie Harbour (>15m) and large-scale die offs of benthic fauna have occurred (Ross et al., 2016). This coincided with a major expansion of salmonid aquaculture and resulted in regulatory actions being implemented that forced a reduction in production in 2016. Furthermore, the companies are currently trialling waste capture devices in the lining of the pens. These devices are likely to significantly and instantaneously reduce the amount of spillover feed available to other species. While this management action is anticipated to reduce deoxygenation by reducing nutrient inputs, it may also incidentally exacerbate resource limitation in *S. acanthias*.

Considering the apparent interdependence between *S. acanthias* and aquaculture, diet is likely to be a significant driver for the population dynamics of the species in Macquarie Harbour. The standing aquaculture biomass in Macquarie Harbour has increased continuously until very recently (Ross and MacLeod, 2017), and it is therefore likely that the amount of spill-over feed also increased, thereby offering an energy rich dietary subsidy for *S. acanthias*. These resources may have allowed the population to grow to previously unsustainable levels in Macquarie Harbour. However, evidence of resource limitation (Chapter 3) could result in high levels of competition and may suggest that the population currently exceeds the carrying capacity of the harbour. Interestingly, access to pellets is likely to be relatively random or reliant on cognition, and not determined by predatory behaviour or fitness, as are natural prey. Thus, competition dynamics in this population will likely be unique, as factors like larger body size would not

confer a direct competitive advantage for prey access but would have higher energetic requirements. Furthermore, the apparent lack of alternative prey in adequate volumes (based on the general lack of non-pellet prey in the diet and high rate of empty stomachs) precludes conventional compensatory responses to competition such as niche partitioning. With the standing pellet biomass no longer increasing, it is conceivable that expansion and regulation cycles in the industry will continue to play a crucial role in the demographics of *S. acanthias* in Macquarie Harbour. Furthermore, if suitable native fauna (e.g. crustaceans, small teleost fish) do not increase rapidly following decreases in aquaculture production, the capacity of the population to return to a smaller stable size could be compromised through decompensation (e.g. Allee effect).

5.4.5. Ecological role

The demographic traits described herein are likely to have been largely shaped by the environmental and ecological conditions of Macquarie Harbour. The general abundance of the species in the system (Lyle et al., 2014) suggests that they have been able to successfully adapt to the environmental conditions of Macquarie Harbour (Chapter 1). This also means that the species is likely to have played an important local ecologic role as an apex predator, although this could now be diminished due to their diet of aquaculture pellets. Thus, this population represents an ideal case study, providing insight into the ways in which elasmobranch species can adapt to diverse habitats and understanding these processes may help predict the limits of the species physiological tolerance to future change.

Squalus acanthias is the most abundant elasmobranch species in Macquarie Harbour but not the only one, as the Maugean skate (*Zearaja maugeana*) is relatively common in shallow areas (<15 m) of the estuary (Bell et al., 2016). Dietary overlap between skates (e.g. *Raja clavata*) and *S. acanthias* has been reported elsewhere (Demirhan et al., 2007). This was not evident in Macquarie Harbour however, where the diet of *Z. maugeana* is dominated by benthic

crustaceans (crabs and shrimps) and there is no evidence of feeding on pellets (Bell et al 2016, Treloar et al 2017). It is plausible, however, that prior to the commencement of aquaculture operations in Macquarie Harbour *Z. maugeana* and *S. acanthias* may have competed for the same resources. The fact that *Z. maugeana* do not appear to feed on spillover pellets may be linked to habitat preference, tending to inhabit the shallow margins (< 15 m) whereas the aquaculture leases are located over the deeper central areas of the harbour (Figure 2.1). In these deeper areas dissolved oxygen levels now commonly reach values below 2 mg/l (Ross and MacLeod, 2017). Oxygenation strategies of modern elasmobranchs show a dichotomy likely originating after selachian (sharks and dogfishes)-batoidean (rays and skates) differentiation (Price Jr and Daiber, 1967). Unlike in skates, oxygen affinity in *S. acanthias* is strongly determined by urea concentration (Schooler et al., 1966). Through captive feeding experiments, it has been shown that *S. acanthias* has very effective internal regulatory mechanisms for urea, even during long-term fasting (Wood et al., 1979). Thus, *S. acanthias* may be better physiologically adapted than the *Z. maugeana* to deal with these challenging conditions.

The bacterial breakdown of organic nutrients introduced by aquaculture can further contribute to the creation of anoxic conditions (Ross and MacLeod, 2017). By feeding on spillover pellets, *S. acanthias* will have a mitigating effect on nutrient enrichment. Given the abundance of the species in the area, this effect is likely to be important for the nutrient budget of Macquarie Harbour. This valuable ecosystem service appears to be exclusive to the species, as other predatory fish like the *Z. maugeana* (Bell et al., 2016) and escapee salmonids (Abrantes et al., 2011) do not appear to feed on pellets often. Given this direct feedback loop between aquaculture and *S. acanthias*, they may be a valuable indicator species in the system now that a demographic baseline for the population has been constructed.

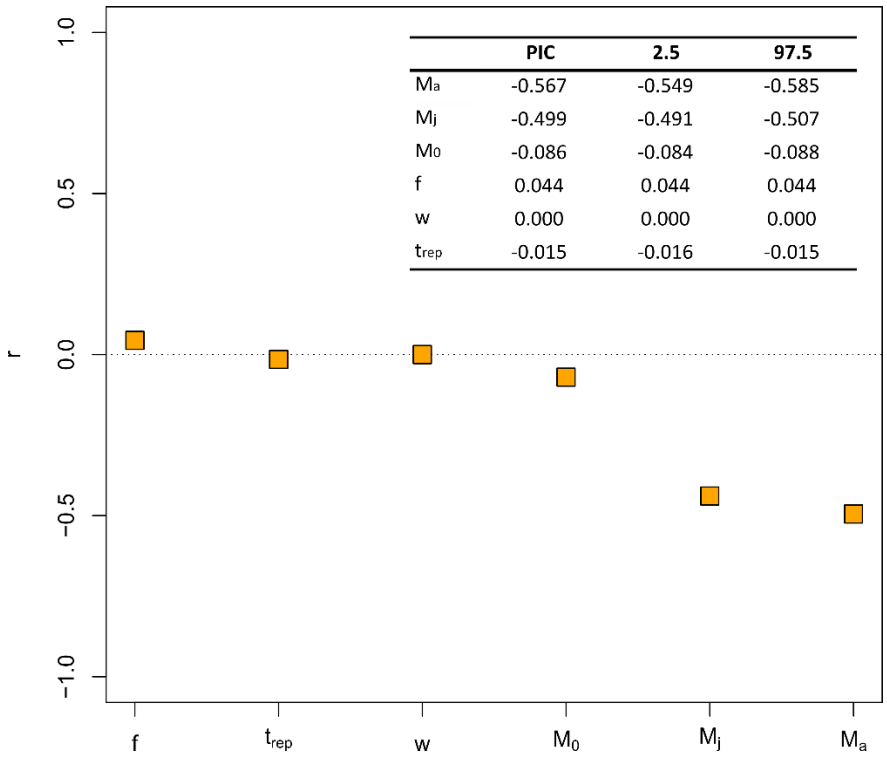


Figure 5.7. Partial inclination coefficients (PIC) for the components of the demographic model. Values represent the relative impact of variability in the initial parameters when the other values remain constant. PIC account for correlation between parameters. Perturbation analysis of the stochastic model conducted using Latin-hypercube sampling.

5.5. FUTURE RESEARCH

This study has described the life history and demography of *S. acanthias* in Macquarie Harbour, allowing the identification of research priorities to help refine management actions (Figure 5.7), as well as new avenues of inquiry to improve our understanding of the mechanisms that may be responsible for the unique characteristics seen in this population. The following is a summary of research questions and priorities arising from this work.

This study revealed several unusual characteristics regarding the reproductive biology of the population, including the possibility of widespread pregnancy failure. Results from demographic modelling revealed these conditions would have immediate and significant negative effects on the population. Therefore, gaining a better understanding of the reproductive patterns in the population and determining if pregnancies are being interrupted, as well as the extent and cause for such a phenomenon, will be essential for the future

management of the population. Reproductive hormone concentrations could be used to provide a quantitative framework for the reproductive seasonality of this population (Awruch et al., 2008), Environmental factors in Macquarie Harbour (e.g. mining runoff, diet, low *in-utero* dissolved oxygen, etc.) may cause epigenetic responses that influence pregnancy and reproduction. Transcriptomics could be used to identify whether genes essential for the regulation of pregnancy are inactive thereby helping to identify what stage of pregnancy is failing and potentially what is most likely to be causing this phenomenon (Pantalacci and Sémon, 2015). Likewise, fatty acid analysis applied to different tissue types (i.e. muscle, liver and gonads) can help detect the effects of heavy metal bioaccumulation (i.e. long-term exposure to mining pollutants, e.g. Hg, Pb) or of inadequate diet (i.e. increased ketone body production due to malnutrition, e.g. ketoacidosis) which may have physiological links to reproductive failure (Wasser et al., 2017).

Identification of the long-term spatial ecology of this population may be impossible through traditional tracking approaches due to their long life-span. Likewise, it is important that age estimates be validated to help corroborate some the results presented in this work. These goals may be achievable through analysis of the chemical composition of calcified structures with accretionary growth such as vertebrae or spines. Calcium is commonly replaced by heavy elements during the calcification process, after which no further reworking of the calcifying matrix occurs (Clement 1992). Osseous microchemistry fingerprinting may be particularly suitable for use as a long-term tracker because the incorporation rate of some elements is determined by environmental concentrations (Smith et al., 2013) and the habitat in Macquarie Harbour (due to mining pollution, water composition and feed composition) is likely to have very distinct chemical properties. By using laser ablation-inductively coupled plasma mass spectrometry (LA-ICP-MS), ontogenetic patterns of feeding and spatial ecology of the population can be determined based on variation in isotopic ratios (Estrada et al., 2006). This will help determine if *S. acanthias* are confined to Macquarie Harbour throughout their life and

especially during juvenile stages which were under-represented in this study. Furthermore, it may be possible to use anthropogenically sourced pollutants as markers for use in stock identification (e.g. mining sourced Pb isotope ratios) and age validation (e.g. appearance of foreign trace elements from pellet diet as a temporal marker).

The possible effects of living in Macquarie Harbour over a multigenerational time scale could be explored through population genetics. This may provide insight into the level of isolation of the population, which will have important consequences in assessing its vulnerability to external pressures. Based on their genetic structure, it has been hypothesized that the *Z. maugeana* population in Macquarie Harbour was introduced into the system during a single colonization event sometime near the last glacial maxima (Weltz., 2018). It is possible that *S. acanthias* colonized Macquarie Harbour under similar circumstances and, if so, some degree of genetic differentiation between this population and other Tasmanian populations is likely to exist. Furthermore, such conditions may have management implications as they could cause a loss of genetic diversity in the population, which has been linked with higher localized extinction risk (McClenachan et al., 2012).

The present study focused on past and future effects that the environmental conditions in Macquarie Harbour have on the life history and demographics of *S. acanthias*. However, the physiological impact of factors such as DO depletion and the long-term effects of a salmonid pellet-based diet remain unstudied. Therefore, it is desirable to assess the anaerobic capacity of the species to help identify maximum tolerance thresholds for low oxygen conditions. Oxygen affinity in the species is linked to plasma metabolites, particularly urea (Price Jr and Daiber, 1967). These in turn are limited by N, which is sourced dietarily (Wood et al., 2010). Therefore, fasting experiments could be used to determine how prolonged starvation may affect the species ability to regulate oxygen flow. Furthermore, diet experiments could be used to elucidate the developmental impacts that a pellet-based diet may have in a long lived marine elasmobranch.

5.6. RECOMMENDATIONS AND GENERAL CONCLUSION

This represents the first detailed assessment of the biology, ecology and life history of *S. acanthias* in Australia. Aspects of the spatial ecology, feeding ecology, reproduction and population dynamics of *S. acanthias* in Macquarie Harbour denote a unique population living, at least semi-permanently, in an unusual habitat. In the absence of historical data for this or other Australian populations of *S. acanthias*, results from this work establish a baseline for future comparisons. By comparing this and adjacent populations, it will be possible to determine whether the demographic characteristics reported herein are unique to Macquarie Harbour or more widely spread throughout Australia. While more research is needed to definitively resolve the long-term spatial ecology of the species in Macquarie Harbour, it would be prudent to treat the population as an isolated stock for management purposes.

Demographic and risk assessment showed that the population has a higher intrinsic rebound capacity than reported elsewhere when reproduction is not compromised. However, due to their restricted distribution, the species is likely to be more susceptible to environmental perturbations than their conspecifics. Thus, past and ongoing anthropogenic impacts in Macquarie Harbour could have considerable effects on the population's well-being, especially those associated with aquaculture. Furthermore, due to their spatial ecology (i.e. high site fidelity without sexual space partitioning), traditional management strategies based on size or spatial protection may be ineffective for this population. Considering this, it is recommended that management action should focus on restoring and maintaining environmental and habitat quality to ensure the permanence of this important and historically ignored species in the area.

There appears to be a strong association between aquaculture and *S. acanthias* in Macquarie Harbour. The species benefits directly through nutritional provisioning while providing an ecosystem service through the consumption of aquaculture waste and helping to mitigate the environmental impacts of the industry to some degree. Recognising this association and

concerns about the environmental impacts of the aquaculture industry, it is feasible that *S. acanthias* could represent an ideal indicator species to monitor overall ecosystem health and recovery and thus should be considered in future impact assessments.

An unexpected finding from this study was the lack of clear spatial segregation between sexes as evidenced by a continuous overlap in core ranges of all individuals. Interestingly, there was evidence of a behavioural avoidance strategy which provides a form of social interaction mediated segregation. Behavioural adaptations, including niche partitioning, range shifts and spatial segregation, to density dependent processes have been documented in several elasmobranchs (Sims, 2003). However, to my knowledge this is the first documented example of an elasmobranch developing an alternative behavioural strategy based on social affinity when habitat restricts the use of traditional spatial partitioning strategies. Social association patterns and behaviour remain largely unstudied in elasmobranchs, but the results of this work show that they may play an important role in the spatial ecology of some species.

Although this work is specific to a single population of *S. acanthias*, the methods and analysis protocols used are broadly applicable for other species. Emphasis was given to the use of multi-model approaches to help account for uncertainty in data-poor conditions and provide a more robust interpretative framework. Studying aberrant circumstances in well understood species like *S. acanthias* can serve as a model for understanding the mechanistic links between life history and environmental change in elasmobranchs. Furthermore, given that Macquarie Harbour has a small geographic extent and is geographically isolated, the system represents an ideal natural laboratory and *S. acanthias* an ideal model species to study the interactions of aquaculture, environmental degradation, and climate change in a long-lived elasmobranch.

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Supplementary Material

Appendix 1

Model Implementation

Utilization distributions

To implement the spatial analysis all detection records were compiled into a unified database using the VEMCO, VUE software. Given that this study was a component in a larger multi-species tracking operation, a data export was created which contained only the records for the transmitters associated with *S. acanthias*. This dataset contained the receiver id, transmitter id and date-time stamp for each unique detection event.

Data were imported into R statistical software for clean-up and preparation. A column with the location coordinates of the listening stations was added to the data using a UTM projection in metres. Records were ordained chronologically, and duplicates deleted. Using the release date of the individuals, the first two days of data for each were filtered out. For each individual, the timestamps were used to calculate the time lag between subsequent detections.

The BBMM model allows for the use of different values of uncertainty around the location error. This can be useful when detection ranges are known to vary between sites or through time. As range testing suggested that the efficiency and range of the array was consistent in Macquarie Harbour, a single measurement of error was chosen (i.e. 405 m).

A potential problem with the use of random walk models is that when the elapsed time between two subsequent locations is too large, the potential area of use will expand considerably. To avoid the over inflation of probabilities introduced by this phenomena, a maximum time lag was set to limit the model. There are no empirical ways to estimate a lag cap; therefore a value must be carefully selected considering the size of the study area, the mobility of the individuals and the design of the acoustic array. For Macquarie Harbour, a value was chosen based on the approximate time it would take an average individual of the species to swim between the two most distal adjacent receivers at an average speed. Additionally, a sensitivity analysis with multiple lag values was conducted to explore the effects on the shape of the UD. When the

maximum lag allowed was very small, the resulting UD appeared similar to a standard KUD, failing to recognize the mobile link between receivers. Conversely, when no limits were set to the maximum lag allowed, the UDs were overinflated and the probabilities were so diffused that the UDs lost any usefulness.

Although UDs are a commonly used analysis tool in elasmobranch movement studies, BBMMs have only been used to analyse large scale migratory movements in a few species. In smaller scale, space utilization studies like this, analysis has been traditionally performed using minimum convex polygons and kernel probability estimations. The use of BBMMs was compared with these methods by creating UDs of the same dataset using all three models. The BBMMs were able to successfully identify connections and transit areas between receiver clusters that the other models failed to recognize. This suggests that the use of BBMMs can yield equal or better results than the other UD calculation techniques.

The calculations were performed in R, using a visual interphase based on the code in the package BBMM (Nelson et al. 2011). For each individual the location and time difference between all subsequent locations was used to calculate a measure of movement variance. Movement variance is a theoretical component of the model but represents a crude characterization of behaviour. The variance was obtained by using an optimization routine on a custom likelihood function and selecting the minimum value from the resulting distribution (Nielson et al., 2011). The UDs are computed using this data by giving each cell in a grid matrix a probability of use.

Any probability assigned to cells representing land areas was removed, and the remaining values re-normalized to 1. The isopleths corresponding to the core home range (i.e. 50%), extended home range (i.e. 95%) and total activity area (i.e. 99%) were calculated for each UD using the contour tool from the raster package in R (Hijmans, et al., 2016).

The resulting matrices were saved as raster files along with the associated spatial metadata. Sex specific UDs were generated by adding the values of each individual raster and re-normalizing to 1.

State transition movement models

To implement the state transition model the MATLAB add-on, SOCPROG, was used. Five broad areas were defined using receiver clusters. An additional column with the area code was added to the detection data and input into SOCPROG. Prior to the analysis, the data were subdivided by sex and season. In this type of analysis, each area is considered a state, and the model evaluates the probability of an individual transitioning to an alternate space at any given moment, regardless of its past states. Movement is assumed to occur as a discrete Markovian process, and the calculations yield a transition probability matrix.

As detection range coverage is not full, it could be possible for individuals to swim through an area without being detected. This could lead to the model assigning a transition probability to non-adjacent areas. To avoid this problem, a maximum lag constraint was implemented for this calculation as well.

The calculations are performed through an iterative process based on starting values provided to the model. This type of analysis is extemporaneous, as it does not incorporate the chronology of transitions between states. Since no individuals left the study area, the system was considered to be closed, implying that all individuals must be at some state at all times. The error range of the individual matrix components was estimated using bootstrapping.

A measurement of the relative importance of the individual areas can be calculated as an index of centrality. This is obtained from the leading eigenvector of the transpose of the transition matrix (i.e. the stable state distribution).

Social interaction networks

Implementation of social network analysis was performed using SOCPROG (see above). Data were arranged into three columns containing the individual id, date-time stamp and receiver id. The receivers were selected as the grouping variable and the sampling period was set to four hours. An initial sampling period of one hr was used, but results did not differ when increased to four hrs, so the latter was chosen to speed computations.

Since detection range coverage was smaller than the study area, a simple association index was used, where only interactions when both individuals in the dyad are identified during the sampling period are counted. The calculations result in an association matrix including all individuals. Additionally, network analysis statistics are automatically calculated to characterize the degree of social differentiation and structure in the population. To determine if statistically significant preferred or avoided associations exist, a permutation test was conducted. To account for sex specific behaviours, we included sex as a grouping variable. The test compares the data to a theoretical population, where associations occur randomly.

Visualization of the social groupings was initially achieved using multidimensional scaling to generate a network diagram. Defined social groupings were hard to define using this method, so a cluster analysis was used instead. A possible problem with clustering techniques is that they are designed to find groupings even when there are none. To avoid this problem, community division by modularity was analysed and for each dendrogram, the maximum modularity was used as a cut-off point for group differentiation.

Network analysis does not factor time into the calculation of sociality. As a way to explore the temporal components of sociality, lagged association indices were calculated. These were compared to the null association indices for each season. The crossover point between the two lines indicates the time after which previous association will no longer affect the likelihood of

future association for any given individual. Additionally, to explore possible differences between sexes, sex specific lag association indices were also calculated for each season.

Results

Table 0.1. Summary tagging information from all individual *S. acanthias* acoustically tracked in Macquarie Harbour.

	Tagging Data				Biological Information		Detection Summary		
ID	Tag type	Location	Release date	Time of release	Sex	Length	First detection	Last detection	Total detections
SD02	V13-1x	Kellys Basin	5/11/2013	17:50	M	577	6/11/2013	6/02/2015	2012
SD04	V13-1x	Kellys Basin	5/11/2013	17:50	M	480	5/11/2013	6/02/2015	1053
SD05	V13-1x	Kellys Basin	5/11/2013	17:50	M	574	5/11/2013	6/02/2015	2195
SD06	V13-1x	Kellys Basin	5/11/2013	17:50	M	559	5/11/2013	6/02/2015	1673
SD11	V13-1x	Liberty Point	6/11/2013	11:00	F	754	6/02/2013	5/02/2015	781
SD12	V13-1x	Liberty Point	6/11/2013	11:00	F	724	6/02/2013	28/01/2015	2151
SD13	V13-1x	Liberty Point	6/11/2013	11:00	F	758	6/11/2013	6/02/2015	4151
SD14	V13-1x	Liberty Point	6/11/2013	12:05	F	768	6/11/2013	3/02/2015	3655
SD15	V13-1x	Liberty Point	6/11/2013	12:07	F	831	6/11/2013	6/02/2015	2408
SD16	V13-1x	Liberty Point	6/11/2013	12:10	F	793	6/11/2013	6/02/2015	2434
SD31	V13-1x	Liberty Point	18/02/2014	16:14	M	500	18/02/2014	2/02/2015	1953
SD33	V13-1x	Liberty Point	18/02/2014	18:10	M	553	18/02/2014	5/02/2015	2546

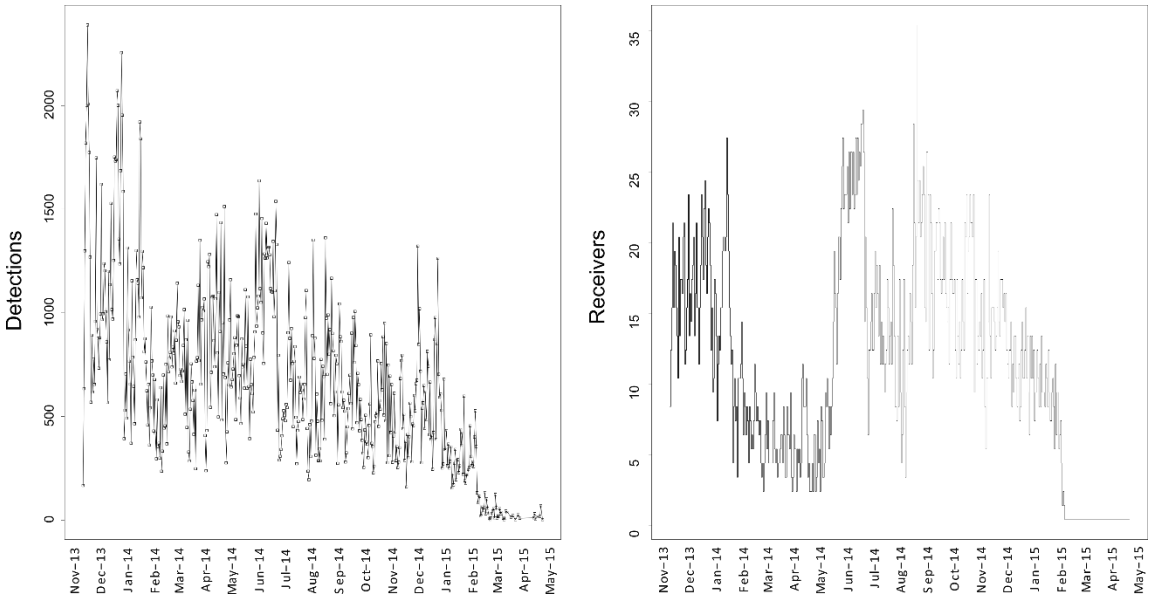


Figure 1. Total daily detections and number of unique listening stations with successful detections of *S. acanthias* in Macquarie Harbour from Nov 2013 to May 2015. Note that dismantling of the acoustic array began in Feb 2015

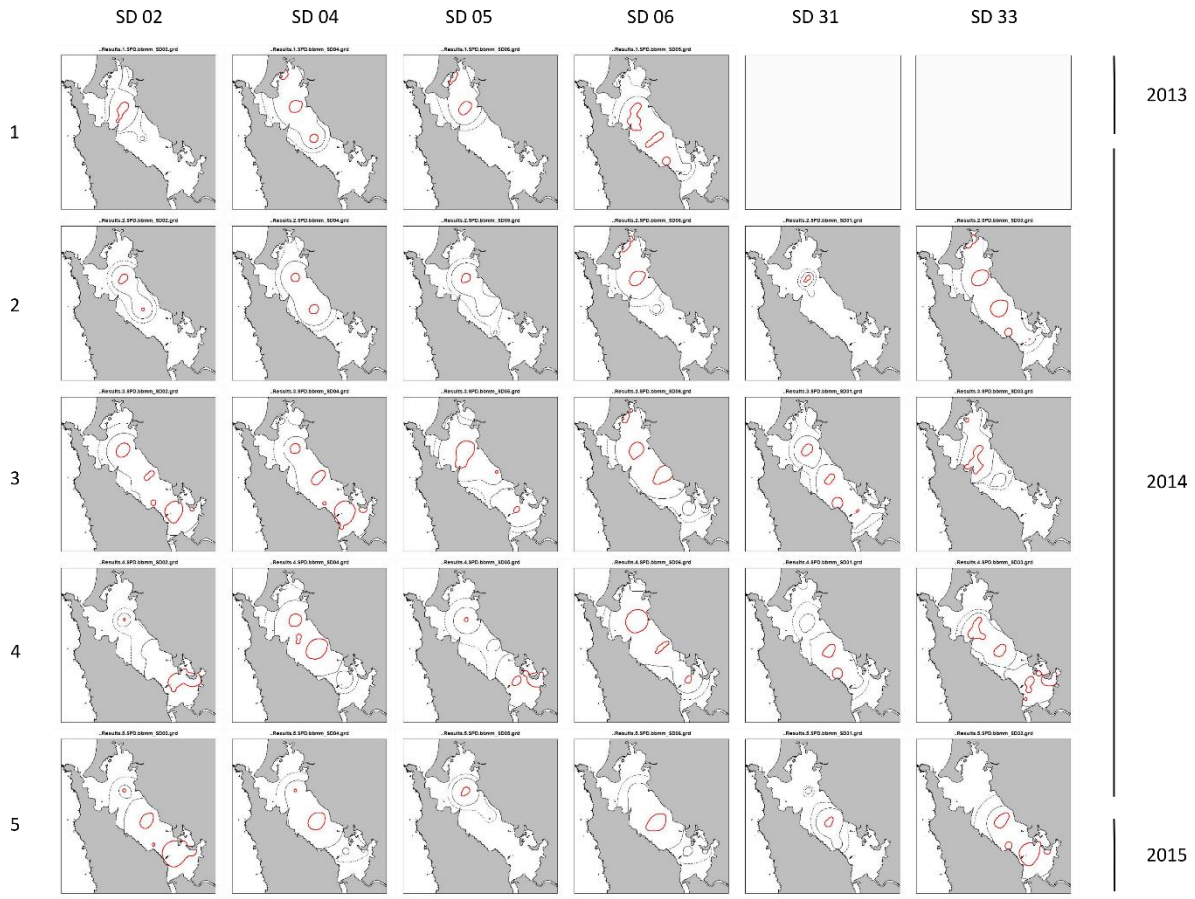


Figure 2. Individual utilization distributions of male *S. acanthias* calculated using a BBMM approach. Contours show core, extended and total home ranges. Each column represents a unique individual, and each row a 3-month sampling period starting in Nov 2013 until early 2015

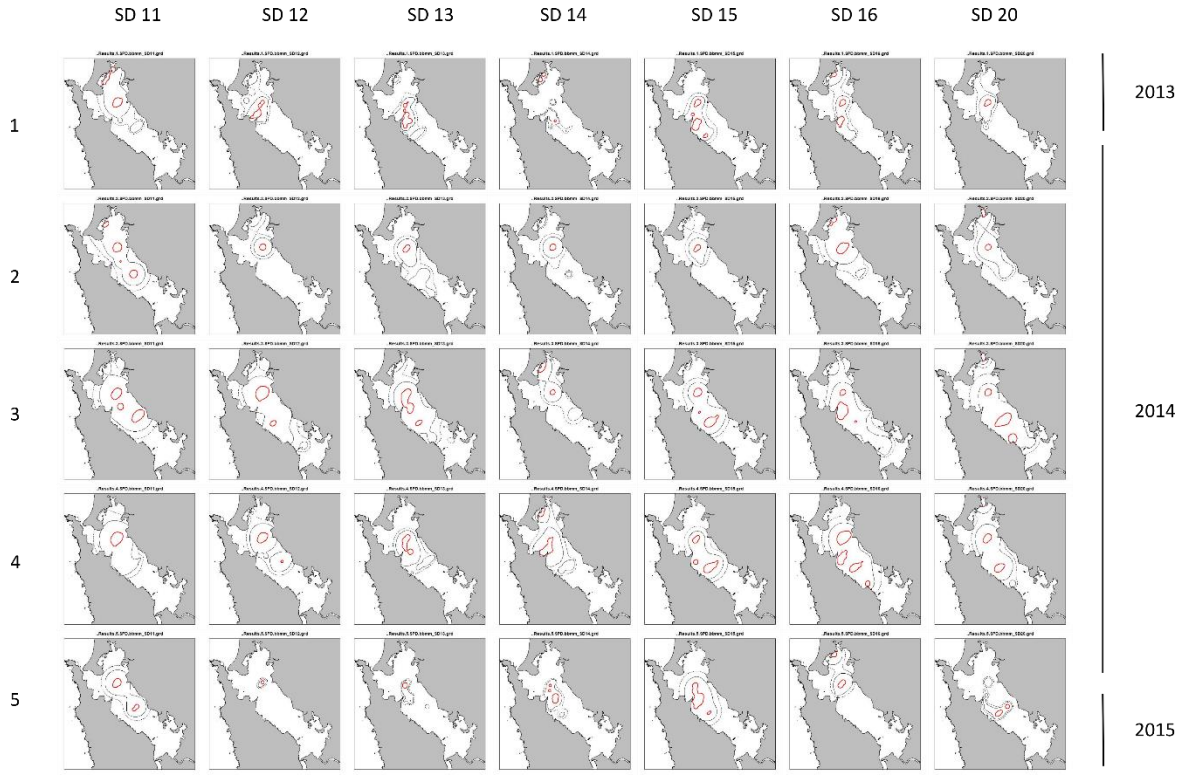
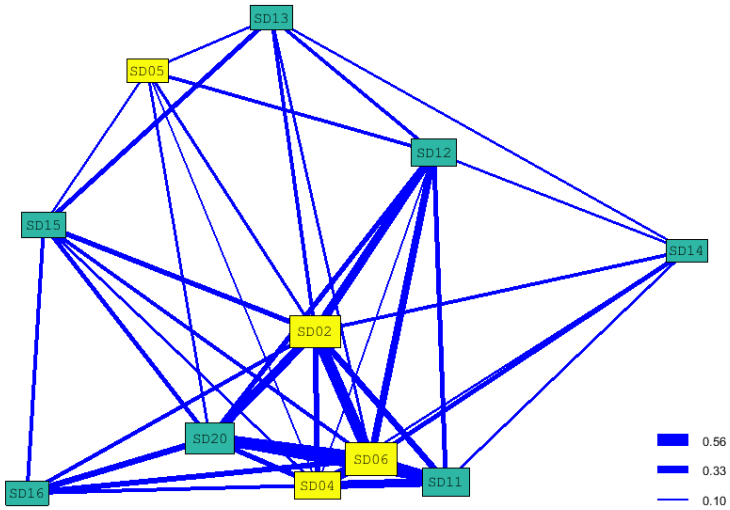
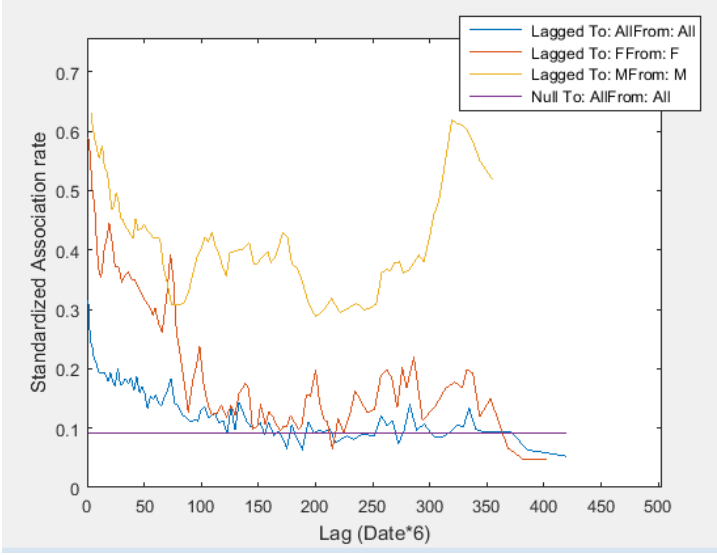


Figure 3. Individual utilization distributions of female *S. acanthias* calculated using a BBMM approach. Contours show core, extended and total home ranges. Each column represents a unique individual, and each row a 3-month sampling period starting in Nov 2013 until early 2015

Summary of social network analysis results

	Test or unit	Season 1			Season 2			Season 3			Season 4			Season 5		
Social network summary		**N**	**t**	**p**	**N**	**t**	**p**	**N**	**t**	**p**	**N**	**t**	**p**	**N**	**t**	**p**
Number of individuals		11.0			13.0			13.0			13.0			13.0		
Number of sampling periods (sp)	sp=4 hrs	445.0			536.0			557.0			555.0			523.0		
Mean ids/ sp		173.4			118.5			130.4			97.9			72.1		
Mean individuals/sp		5.1			4.1			4.6			4.5			3.5		
Mean individuals per sp	%	45.9			31.4			35.6			34.7			27.2		
Mean associations per dyad		18.6			33.2			12.9			7.7			5.5		
Generalized affiliation index	AI = both identified	0.7			0.8			0.6			0.9			1.8		
Association between /withis sexes	Mantel's test		-1.27	0.10		0.53	0.70		1.60	0.94		2.55	0.99		1.41	0.92
Network statistics			**All**	**Males** **Females**		**All**	**Males** **Females**		**All**	**Males** **Females**		**All**	**Males** **Females**		**All**	**Males** **Females**
Gregariousness	%		0.20 (0.08)	0.24 (0.1) 0.18 (0.6)		0.54 (0.1)	0.56 (0.11) 0.53 (0.11)		0.16 (0.06)	0.19(0.04) 0.19(0.08)		0.13 (0.06)	0.1(0.03) 0.15(0.07)		0.16 (0.07)	0.16 (0.04) 0.16 (0.1)
Mean estimated group size			3.04(0.81)	3.45 (1.03) 2.81(0.62)		7.54 (1.26)	7.68 (1.29) 7.41(1.32)		3.25(0.75)	3.26 (0.52) 3.24 (0.94)		2.25 (0.68)	2.2 (0.34) 2.78 (0.8)		2.9 (0.9)	2.89 (0.48) 2.9 (1.19)
Network diagrams		**N**	**sd**		**N**	**se**		**N**	**se**		**N**	**se**		**N**	**se**	
Non metric multidimenssional stress	(%)	3	0.09		2	0.24		2	0.12		2	0.16		2	0.13	
Hyerarchical agglomerative clustering		**N**			**N**			**N**			**N**			**N**		
Max modularity from gregariousness		0.114			0.084			0.097			0.199			0.369		
Association index at max modularity		0.175			0.527			0.152			0.173			0.113		
Monte carlo- permutation test for associations for individulas				**p**			**p**			**p**			**p**			**p**
SD prefered associations.				0.001			0.001			0.04			0.001			0.001
SD of non-zero elemants (avoided)				0.001			0.001			0.04			0.001			0.001
Monte carlo- permutation test for associations for individulas																
Prefered by sex				0.001			0.001			0.001			0.002			0.001
Avoided by sex				0.001			0.001			0.001			0.002			0.001
SD of group size (gregariousness)				0.385			0.073			0.500			0.576			0.134


SD13
SD05
SD12
SD15
SD14
SD02
SD20
SD06
SD16
SD04
SD11
0.56
0.33
0.10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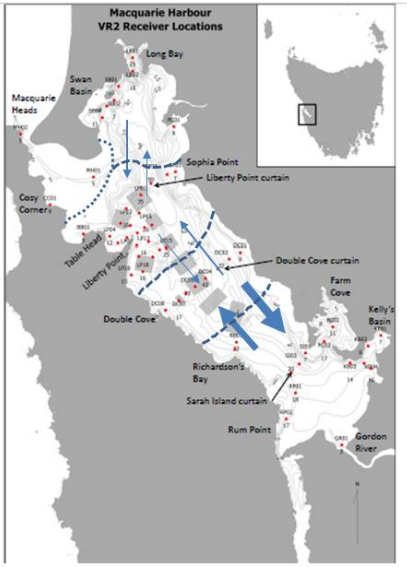
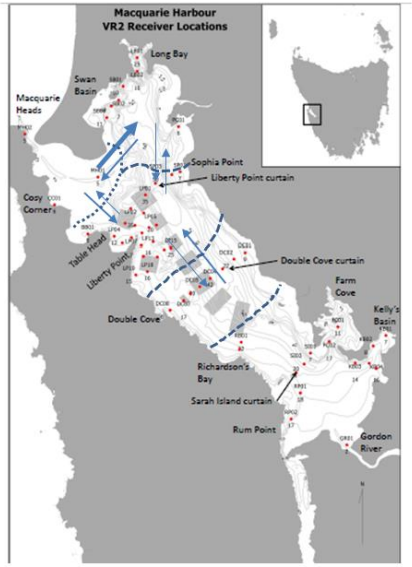
Lagged To: FFrom: F
Lagged To: MFrom: M
Null To: AllFrom: All
Standardized Association rate
Lag (Date*6)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0
50
100
150
200
250
300
350
400
450
500


Macquarie Harbour
VR2 Receiver Locations
Long Bay
Swan Basin
Macquarie Heads
Sophia Point
Liberty Point curtain
Cosy Corner
Table Head
Liberty Point
Double Cove curtain
Farm Cove
Kelly's Basin
Double Cove
Richardson's Bay
Sarah Island curtain
Rum Point
Gordon River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Sarah Island curtain
Rum Point
Gordon River

Appendix 2

Morphometrics and sample details

Table 0.2. Size composition (TL mm) of *S. acanthias* used in this study from multiple locations in Tasmania

Site	Males			Females		
	n	$\bar{x}$	min-max	n	$\bar{x}$	min-max
Macquarie Harbour	278	669	464–731	163	859	369–985
South-East Tasmania	31	618	521–739	10	581	554–619
Bathurst Harbour	81	607	342–632	3	440	370–527
Macquarie Harbour *	245	658	539–790	163	901	507–1013

*Lyle et al. (2014). Data from 2011 excluded because sex was not reported

Table 0.3. Regression coefficients for morphometric conversions in *S. acanthias* derived from individuals in Macquarie Harbour – lengths in mm and weight in g.

Length - Length		Linear regression coefficients				
		n	α	β	R^2	p
♂+♀	FL to TL	147	8.76	1.11	0.99	<0.005
♂+♀	PCL to TL	147	31.54	1.56	0.98	<0.005
♂	FL to TL	67	9.22	1.11	0.98	<0.005
♂	PCL to TL	67	69.42	1.46	0.90	<0.005
♀	FL to TL	80	12.1	1.11	0.99	<0.005
♀	PCL to TL	80	35.57	1.56	0.98	<0.005

Length - Weight		Logistic regression coefficients				
(TL) - (TW)		n	α	β	R^2	p
♂+♀	Combined sexes	188	1.21E-07	3.53	0.94	<0.005
♀	Female	90	1.28E-07	3.52	0.95	<0.005
♂	Male	98	1.75E-06	3.11	0.96	<0.005

Appendix 3

Model implementation and data analysis

Demographic Modeling

Demographic analyses were conducted using life history tables and age-structured Leslie Matrices to calculate population statistics and reference points based on fecundity (*f*) and mortality (M) life history parameters. Both approaches are single-sex models focusing on females and produce nearly identical results. Life history tables use a discrete version of the Euler (Lotka) equation which can be solved iteratively to calculate the intrinsic growth rate of a population (r):

$$1 = \sum_{x=1}^{w} l_x m_x e^{-rx}$$

where *w* is the maximum reproductive age, m_x is the number of female offspring produced annually by an individual (i.e. *litter size* x *sex ratio/ reproductive periodicity*) and l_x is the probability that an individual will survive to age x :

$$l_x = l_{x-1} * e^{-Mx}$$

Similarly, in age structured Leslie Matrix, age specific survivorships ($S_x = e^{-Mx}$) and age specific fecundity (f_x) are used to construct a projection matrix (Cortes 2002). Fecundity was calculated as $f_x = S_{x-1} * m_x$, when $x > t_{rep}$ (age at first reproduction). Matrix algebra can be used to find the leading eigenvalue (λ), which represents the final rate of population growth. The relationship between r and λ can be described by the function (Simpfendorfer 2004):

$$\lambda = e^r$$

The left and right eigenvectors of the matrix represent the population's reproductive value (v) and stable age distribution (w) respectively.

Demographic reference points were derived from these models. The net reproductive rate (R_0) is the mean number of offspring produced by an individual through its life:

$$R_0 = \sum_{x=\alpha}^{w} m\, l_x$$

Generation time (G) is the average length of time between the birth of an individual and the birth of its first offspring:

$$G = \frac{\sum_{x=\alpha}^{w} l_x m_x x}{R_0}$$

Matrix Elasticities

Elasticities are a scale-less metric of the relative effect perturbations in the individual matrix elements will have on λ while holding all other elements constant (Caswell 1989). They can be calculated from the projection matrix as:

$$e_{ij} = \frac{a_{ij}}{\lambda} \frac{v_i w_j}{\langle \boldsymbol{w} | v \rangle}$$

where a_{ij} is the matrix element in row i and column j, v is the reproductive value vector, w is the stable age distribution vector and <**w**,v> is the scalar product of vectors v and w. Matrix elasticity elements where summed to represent fecundity, juvenile survival and adult survival.

Intrinsic Rebound Potential

Intrinsic rebound potentials represent the level of r given a mortality level chosen for maximum sustainable yield (MSY). Unlike the standard deterministic life history tables, this method incorporates concepts of density dependent compensation, where survival increases as population size diminishes. Two values of M have been proposed to simulate MSY in elasmobranchs, 2M and 1.5M (Smith et al., 1998). To calculate r_{2M}, density-dependent compensatory effects are assumed to occur as an increase in pre-adult survival (l_α). The Euler

Lotka equation is used to find l_{α} at stationary equilibrium (r=0) when mortality is increased (Z=M+F=2M):

$$e^{-Z}+l_{\alpha Z}{}^{m}\,[1-e^{-Z(w-\alpha+1)}] = 1$$

Once $l_{\alpha,Z}$ is known, F is removed again so that Z=M. The intrinsic rebound potential can be calculated solving the Euler Lotka equation for r. The resulting metric (r_{2M}) is an integrative measure of growth potential that can be used to compare multiple species.

Analytical reference points

Stock assessment requires knowledge about optimal depletion levels that are often unknown in data-poor fisheries. In these situations, a proxy is commonly used in lieu of inestimable MSY levels. One common proxy is based on spawning percentage ratio (SPR) (Goodyear 1977). Brooks et al, (2009) proposed a method to derive SPR reference points analytically from life history data. SPR at maximum excess recruitment (MER) is the optimal depletion level of spawners in a stock-recruitment function. SPR_{MER} can be calculated for the Beverton-Holt function based on the maximum lifetime reproductive rate at low density (ά) in the absence of fishing. This can be calculated directly from the life history table, so that:

$$SPR_{MER} = \frac{1}{\sqrt{\acute{\alpha}}}$$

It is also possible to calculate SPR_{crash}, which is the SPR as level of spawners approaches the origin, beyond which, the population would be extinct. The compensation rate and SPR are inversely correlated, therefore,

$$SPR_{crash} = \acute{\alpha}^{-1}$$

<u>Monte-Carlo Simulation and perturbation analysis</u>

To generate the stochastic modelling framework, Monte-Carlo simulations were used where the value of the initial parameters was changed at each iteration. Each of the initial parameters was

chosen from a probability distribution for each individual trait. The values and error coefficients used were taken from Chapters 3 and 4 of this thesis (Table 5.2). The growth coefficients from the VBGF were modelled as a normal distribution with a multivariate error calculated from the multi model framework presented in chapter 4. Age at maturity was also assumed to be normally distributed. Age specific fecundity and natural mortality were considered to have a triangular distribution. Maximum age was given a uniform distribution between the maximum observed age and a theoretical longevity estimate (as per Mollet et al, 2002), $t_{max}= 7\ \ln(2/k)$. A total of 10,000 simulations were performed, resulting in a distribution of results of the models, elasticities and reference points.

To analyse the individual effect that variation of each of the individual parameters had on the model results, correlation coefficients were calculated for fecundity, age at reproduction, maximum age and mortality of adults, juveniles and fecundity. These values are not independent; therefore, the use of multiple linear regression was inadequate. Instead, the coefficients of the slope (partial inclination coefficients) were calculated using a Latin-hypercube sampling technique (Chalom et al., 2013).

Risk analysis

To explore the effects of increased mortality, the Monte Carlo simulations were re-run with an added fishing mortality parameter. The value of F went from 0 to 1 and was increased in 0.05 increments after every iteration of the simulation. To analyse the effects of length selectivity, knife-edge entry into the fishery was simulated at increasing ages at first capture (AAFC). This resulted in a matrix of λ values at different levels of F and AAFC, which was used to calculate F_{crit}. F critical is defined as the maximum harvest level at stable equilibrium (λ=1).